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THE PATHOGENESIS OF ENAMEL HYPOPLASIA: AN EXPERIMENTAL STUDY[1]

SEYMOUR J. KRESHOVER[2]

Yale University, New Haven, Conn.

In a previous publication (1) the author described changes in the developing teeth of a series of infants and human fetuses which permitted the tracing of the pathogenesis of enamel hypoplasia from its earliest manifestations to the end result of clinically demonstrable defects. The present study was undertaken in order to provide an experimental check on the conclusions drawn from the findings in human teeth.

Although hypoplastic lesions have been produced by Erdheim (2) and Toyofuku (3) in parathyroidectomized rats, and by Mellanby (4) in puppies fed a rachitogenic diet, no attempt was made to demonstrate a complete histogenesis of enamel hypoplasia. Schour and Smith (5), however, in an experimental study of fluorisis in rats, endeavored to find the earliest lesions that could be detected histologically. Attempts to demonstrate a complete sequence of histologic changes in the pathogenesis of experimentally produced enamel hypoplasia, present certain difficulties. Available methods of study must, therefore, be assayed before one or another procedure is followed.

The continuous development and growth of the incisor teeth of rodents makes these animals particularly desirable as subjects for study. Manifestations of hypoplastic lesions can thus be examined in the same tooth at all stages of enamel formation. The end result of injury to the functioning ameloblasts in the apical portion of the tooth would be manifest in the incisal region where enamel has reached its completed formation stage. To successfully accomplish this end the ameloblastic injury must be such as to result in clinically demonstrable defects. However, if this be done, the severity of the initial lesion and the rapidity of its progression make it problematical whether all the typical changes in the histogenesis of enamel hypoplasia would be demonstrable. That is, there may be so rapid an alteration of ameloblastic function and morphology that the visible changes would be more toward the end result of abnormal enamel formation. Sacrificing animals at varying periods of time following application of the injurious agent might conceivably provide a method for revealing certain stages of enamel hypoplasia at their moment of manifestation.

[1] The data presented are taken from a dissertation presented to the Faculty of the Graduate School of Yale University in candidacy for the degree of Doctor of Philosophy. This work was done, in part, during tenure of a Carnegie Fellowship from the Thomas W. Evans Museum and Dental Institute, School of Dentistry, University of Pennsylvania. Received for publication April 20, 1944.

[2] Captain D.C. AUS.

A more feasible experimental procedure would be to vary either or both of the following factors: the type of etiologic agent and the susceptible host. It is apparent that two such variables could make for a diversity of resulting lesions, the severity of which would depend upon the degree of susceptibility of a particular animal specie to a particular injurious agent. It would, therefore, be within the realm of possibility to prognosticate the severity of injury to the developing tooth. Individual variations in susceptibility of animals of the same species to identical injurious agents is, however, an additional consideration.

TABLE I

ANIMAL NUMBER	WEIGHT OF ANIMAL	INJECTIONS OF TUBERCLE BACILLI				EVIDENCE OF TUBERCULOSIS AT AUTOPSY		ALIZARINE RED S INJECTED			
		Amount	Site of Inocul.	Time between Inocul. and Death		Gross	Micro.	Amount	Number of Injections	Time between Injections	Time between last Injec. and Death
				Sacrif.	Died						
	gm.	*mgm.*		*days*	*days*			*cc.*		*hrs.*	*days*
1	350	0.1	Groin	80		+	+	1.0	6	48—46—48—48—72	72
2	350	0.1	Groin	80		+	+	1.0	6	48—48—48—48—72	72
3	350	0.05	Groin	80		+	+	1.0	6	48—48—48—48—72	72
4	195	0.05	I.P.	71		+	+				
5	350	0.05	Groin	24		+	+				
6	350	0.1	Groin	81		+	+				
7	350	0.05	Groin		8	0	0	1.0	5	48—48—48—48—48	3
8	350	0.05	Groin		9	0	0	1.0	6	48—48—48—48—72	1
9	350	0.05	Groin		10	0	0	1.0	6	48—48—48—48—72	2
10	350	0.1	Groin		13	0	0	1.0	6	48—48—48—48—72	5
11	115	0.1	I.P.		17	0	0				
12	120	0.1	I.P.		24	0	0				
13	350	0.05	Groin		94	+	+				
14	350	0.1	Groin		160	+	+	1.0	6	48—48—48—48—72	152

The aim of the present study was directed toward the attempted production of, first, the earliest stages and, second, the terminal stages of enamel hypoplasia.

Two possible experimental methods were considered for the demonstration of the earliest histologic changes: to employ either a mild injurious agent or to select animals with a relatively high degree of resistance to a particular agent. The low degree of susceptibility of mice to experimental tuberculosis seemed to favor the possibility of such a procedure resulting in dental abnormalities which would be mild in character; that is provided this disease could cause an enamel hypoplasia (6). The probability that an animal with a higher degree of suscepti-

bility to tuberculosis than the mouse would show far more severe dental changes, seemed to afford the means for studying the advanced stages of enamel hypoplasia. The guinea pig was selected for this purpose. No such approach to this type of study has been found in the literature.

MATERIALS AND METHODS

The material and experimental procedure employed in the first part of this study have been described in a preliminary report dealing with tuberculosis as an etiologic factor in abnormal amelogenesis (6). For the purpose of comprehensive presentation, this information is repeated here in part.

A total of 92 mice were used. Seventy of the animals were inoculated intraperitoneally with either the bovine or human type of tubercle bacilli. The ages of the mice at the time of inoculation varied from 10 to 50 days, and the amounts injected ranged from 0.05 to 0.7 mgm. per 100 grams of body weight. The animals were sacrificed at from 186 to 204 days after inoculation. The 22 mice which served as controls were sacrificed at corresponding age periods.

Following celloidin embedding, decalcification and paraffin infiltration, the mandibles of all 92 animals were cut serially in a longitudinal plane, mounted, and stained with hematoxylin and eosin. Some of the maxillae were similarly prepared.

The teeth of 20 guinea pigs completed the material for the present study. Fourteen of the animals were inoculated, either intraperitoneally or in the groin region, with the human type of tubercle bacilli. The remaining guinea pigs served as controls. The dosages, site of inoculation, and duration of the experiment are given in Table I. Intraperitoneal injections of Alizarine Red S were given in some instances. All the animals were sustained on normal, adequate diets. A complete autopsy was performed in each case immediately after death. The mandibles were removed, divided into right and left halves, and fixed in 10% formalin. After following the same histologic technique of double embedding as employed with the mouse material, longitudinal serial sections were cut at 8 micra and stained routinely with hematoxylin and eosin.

FINDINGS

Since a detailed description of the dental changes in the series of tuberculous mice has been made previously (6), only such findings as bear upon the purpose of this report will be presented.

At autopsy 69 of the total of 70 experimental animals showed evidence of tuberculosis in their viscera. A like number manifested injury to the developing dental organ. The negative findings were not in the same animal. No changes were observed in the control group.

The dental lesions were strikingly similar to those described in human material (1) as representing the earlier stages in the pathogenesis of enamel hypoplasia. Typical were, the deposition of enamel-like droplets subjacent to injured enamel forming cells, pyknosis and karyolysis of the nuclei, and vacuolization of the ameloblastic layer. These changes are demonstrated in *figs. 1, 2* and *3*.

Of the guinea pigs inoculated with tubercle bacilli, 11 showed marked changes

in the ameloblasts and subjacent surface of organic enamel matrix. No morphologic change was demonstrable in the ameloblasts of the remaining 3 experimental animals. In many instances, the dentin showed evidence of developmental disturbance. The 6 animals comprising the control group showed no dental abnormalities.

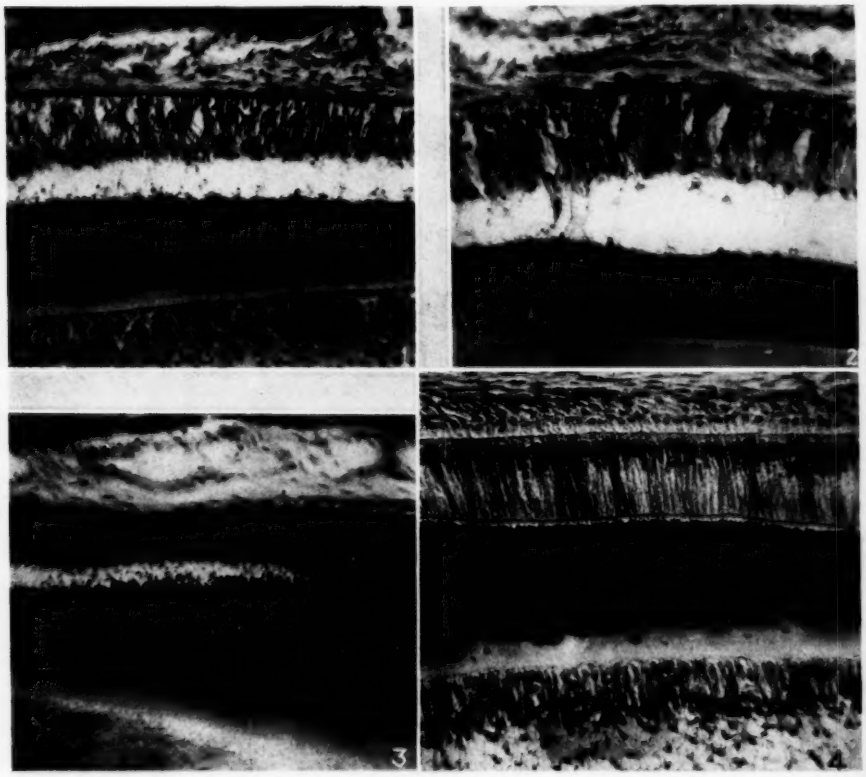


Fig. 1, 2 and 3. Mandibular incisors of tuberculous mice showing ameloblastic injury and deposition of enamel-like droplets. (Orig. mag. × 225; × 290; × 200)

Fig. 4. Normal appearance of ameloblasts and organic enamel matrix as seen in a control guinea pig. (Orig. mag. × 270)

Routine autopsy examination disclosed gross evidence of tuberculosis in the viscera of 8 animals of the experimental group. Three of these guinea pigs failed to show any changes in the enamel organ. Certain abnormalities were observed, however, in the dentin. Although no microscopic lesions of tuberculosis were found in the remaining 6 experimental animals, all showed definite injury to the ameloblasts. Stains for acid fast organisms were negative in all 6 cases.

The characteristic dental changes are best recognized when compared with the normal picture seen in the control animals (*fig. 4*). The tall, slender ameloblasts are regular and uniform in arrangement, and their elongated, pale blue staining

nuclei are located in the usual peripheral position. The ameloblastic basement membrane is well defined and the underlying surface of organic enamel matrix shows the usual uniform terminations of the enamel rods. The stratum intermedium is composed of several layers of cuboidal cells, and the stellate reticulum of loosely arranged epithelial cells connected by long intercellular bridges.

The dental enamel changes observed in the experimental animals are uniformly severe and consequently permit comparison with those lesions described in human teeth (1) as representing the later stages in the pathogenesis of enamel hypoplasia. The least severe of these changes is characterized by a complete alteration in the appearance of the ameloblastic layer (*fig. 5*). Ameloblasts are no longer recognizable, and in their stead are numerous, haphazardly arranged, pyknotic and karyolytic nuclei, and cytoplasm which has been reduced to a faint, pink staining, amorphous structure. Scattered throughout are countless, small, round, blue staining droplets having the appearance and staining characteristics of calcium. Not illustrated in the photomicrograph, is the extension of these tiny globules into the reduced outer enamel epithelium and outer stroma of mesenchymal and young connective tissue. More frequently, however, the droplets are of larger size and are present only in the zone of ameloblastic injury. In many instances, the globular material is in the form of large, irregular masses probably formed by the fusion of many small droplets (*fig. 6*).

Possibly representing a more severe change is the transformation of the ameloblastic zone into an edematous-like structure (*fig. 7*). An interlacing network of fine, pink staining fibrils provides the framework for the edema fluid. Numerous fragmented cells and pyknotic nuclei are found throughout the area. In other sections, enamel-like globules of irregular size and shape also are present (*fig. 8*). It is apparent that these globules descend from the region of the stratum intermedium, coalescing as they approach the enamel surface to form larger and more irregular masses.

Lesions similar to those described were observed also in the molar teeth. *Fig. 9* shows an example of one such change. The ameloblastic zone is edematous and contains numerous injured cells. Resting partly upon the enamel matrix surface is a large mass of enamel-like material. Projecting from it are several long strands of similar material which course between the malformed cellular elements, and also toward the enamel surface where they form button-like caps.

The terminal stage of enamel hypoplasia, characterized by a complete cessation of enamel formation following destruction of ameloblasts, is represented in *figs. 10* and *11*. No ameloblasts or remnants thereof are present, and the basement membrane of the stratum intermedium is in direct contact with the underlying thin shell of irregular enamel matrix (*fig. 10*). To the right and left of this wide, cup-shaped defect, ameloblasts are seen to overlie a more completely formed enamel matrix. A similar but more striking change is seen in *fig. 11*. Localized areas of organic enamel matrix extend into the field of functioning ameloblasts forming nodule-like projections. The intervening enamel, underlying completely destroyed ameloblasts, is deficient in amount.

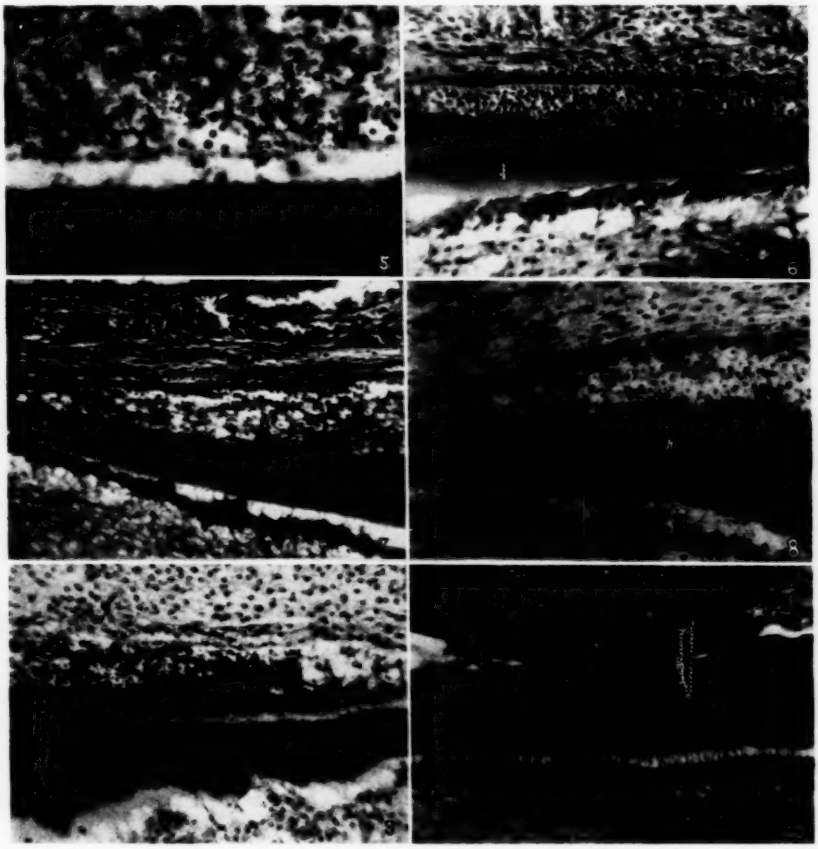


Fig. 5. Mandibular incisor of tuberculous guinea pig showing marked injury to ameloblastic layer and presence of numerous small, round, blue staining droplets throughout region. (Orig. mag. × 720).

Fig. 6. Large, irregular, enamel-like masses overlying the organic enamel matrix of mandibular incisor. Ameloblastic nuclei are pyknotic and irregularly distributed. (Orig. mag. × 375)

Fig. 7. Mandibular incisor of tuberculous guinea pig showing edematous-like appearance of ameloblastic layer. (Orig. mag. × 285)

Fig. 8. Large, irregular masses of enamel-like material are seen in edematous zone between stratum intermedium and organic enamel matrix. (Orig. mag. × 345)

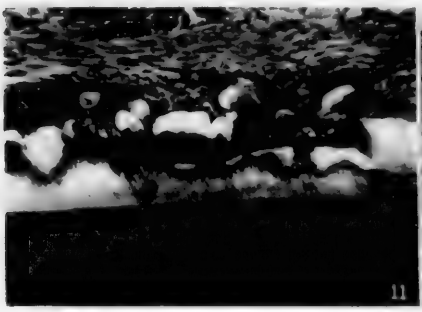
Fig. 9. Molar tooth of tuberculous guinea pig with changes similar to those in *figs.* 7 and 8. (Orig. mag. × 300)

Fig. 10. Cup-shaped enamel defect in mandibular incisor. Note absence of ameloblasts. (Orig. mag. × 200)

In several sections, large cystic areas were discovered in the extreme apical portion of the developing teeth (*fig. 12*). A thin shell of organic enamel matrix forms a portion of the inferior cyst wall, the remainder of the lining being formed by a thick layer of pyknotic ameloblasts.

In addition to the lesions described in the enamel organ, definite changes were observed in the dentin. The most striking of these was the presence of wide, prominent incremental bands signifying long periods of arrested development. In many cases, the odontoblastic layer in the radicular portions of the teeth was absent, and the subjacent pulp contained large, irregular masses of pink staining osteoid tissue in which tubules were frequently seen. In those regions where odontoblasts were present they showed marked cystic changes.

A further interesting change was the unusual staining reaction of the dentin. Whereas normally the predentin layer takes an extremely pale eosin stain, and the calcified dentin a deeper pink or purple stain, the tuberculous guinea pigs often showed a reversal of these staining properties.



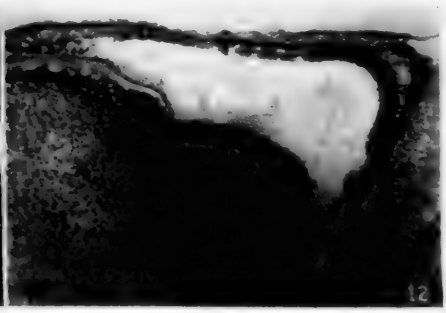


Fig. 11. Multiple notch-like defects in organic enamel matrix of a mandibular incisor. (Orig. mag. × 230)

Fig. 12. Large, cyst-like formation in the apical region of a mandibular incisor from a tuberculous guinea pig. (Orig. mag. × 175)

DISCUSSION

The similarity of changes described in the present report to certain of the lesions observed in human material (1) makes possible a more complete experimental confirmation of the proffered concept regarding the sequence of histologic changes involved in the pathogenesis of hypoplastic enamel.

A discussion of the observed changes in the group of experimental mice (6) is repeated here in part.

Two possibilities are considered which may account for the relatively slight degree of hypoplastic change. Since none of the experimental mice show evidence of incomplete enamel formation subjacent to the injured ameloblasts or gross defects in the erupted portions of the teeth, it is possible that the animals may have been sacrificed before the end result of cell destruction could manifest itself. However, the period of time between inoculation and death (186 to 204 days) seems ample for such defects to make their appearance. It is likely, therefore, that the degree of cell injury is not severe enough to result in cessation of enamel formation. If the injury is not so severe as to cause complete cell destruction, it is conceivable that the enamel forming cells may return to normal function leaving merely a prominent incremental line to mark the period of abnormal amelogenesis. Since mice are comparatively resistant to experimental tuberculosis (7), it is to be expected that even large doses of tubercle bacilli may cause little or no disturbance. It is of interest to note that there is no correlation, in the presented material, between the degree of ameloblastic injury on the one hand and the dosage or duration of the disease process on the other.

Mice receiving small doses of tubercle bacilli show no less severe dental changes than those receiving large doses, and, similarly, animals sacrificed 186 days after inoculation present no significantly different picture than those with a longer disease course.

Of the 14 guinea pigs inoculated with tubercle bacilli, 8 showed gross evidence of tuberculosis in their viscera. Autopsy examination of the remaining 6 animals failed to show such lesions either grossly or microscopically. Since the members of the latter group died in from 8 to 24 days following inoculation, the possibility is considered that the duration of the disease was not sufficient to result in pathologic manifestations. The positive dental findings in these 6 animals cannot, therefore, be definitely attributed to tuberculosis. It is seen in Table I, that 4 of these guinea pigs were given several injections of Alizarine Red S. It is thus possible that toxic effects of the dye may have been responsible for their death as well as for the dental lesions. However, the dye was also given to 4 animals in the group showing gross evidence of tuberculosis at autopsy. One of these guinea pigs survived for 160 days, whereas the remaining 3 were sacrificed in 80 days. This would seem to provide some evidence against the toxic effects of alizarine since identical doses were given in similar number to animals of approximately the same age and size. However, the alizarine injections were discontinued, in all instances, after a brief period of time. Consequently, no positive statements can be made other than that individual variations in susceptibility is a possible factor for consideration.

The 3 animals which failed to show any dental lesions were all from the group of 8 which developed gross signs of tuberculosis in their viscera. Two of these guinea pigs were sacrificed at 80 days and had received alizarine, and the remaining animal was sacrificed at 24 days after inoculation and had not received any alizarine. No attempt is made to explain the absence of ameloblastic injury in these animals. All 3, however, showed evidence of abnormal dentin formation.

CONCLUSIONS

Experimental findings are presented which demonstrate and clarify the sequence of histopathologic changes involved in the pathogenesis of enamel hypoplasia. These changes consist of:

1. Abnormal formation or secretion of enamel-like substance subjacent to and within the cell cytoplasm.
2. Beginning vacuolization of ameloblasts.
3. Complete cystic destruction of ameloblasts.
4. Cessation of enamel formation resulting in notch-like defects.

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ULTRAMICROSTRUCTURES OF THE HUMAN TOOTH AS REVEALED BY THE ELECTRON MICROSCOPE[1]

CHARLES H. GEROULD

The Dow Chemical Company, Midland, Mich.

INTRODUCTION

The field of electron microscopy has expanded considerably within the last few years due in large part to the advances in the application of the transmission electron microscope to surface studies of electronically opaque bodies. Several techniques of applying the electron microscope to the study of surface structures have been developed (1), one of which is the polystyrene-silica surface replica technique of Heidenreich and Peck (2, 3). This replica technique has given excellent results in studying the surface of metals, minerals, glasses, etc., and initial results with tooth structures have indicated that the technique is applicable here (*Fig. 1*. Original magnifications 4500.). Briefly the technique consists in the preparation of first, a negative replica of the surface using a molded thermoplastic polystyrene, and second, a positive replica film by evaporation of silica on the polystyrene molding. This silica film serves as the object viewed in the electron microscope.

The application of this replica technique in the study of tooth structures will be considered in this paper, with the histological interpretation of the pictures so obtained left to a future paper.

THE ELECTRON MICROSCOPE

The RCA, Type B, transmission electron microscope was used in this work. This type of instrument differs chiefly from the ordinary light microscope in that the visible light has been replaced by an electron beam whose wavelength is 1 100,000 of that of light. This removes the principal resolution limiting factor existing in the light microscope and thereby allows a resolution 100 times greater or down to about 0.003 microns, or perhaps somewhat less. Glass lenses cannot be employed to form images with the electron beam, but suitable magnetic or electric fields will serve to attain the desired electronic images. Descriptions of electron microscopes in the literature are numerous (1, 4, 5) so details will not be presented here.

Since electrons are readily scattered and absorbed by matter, bulk specimens, to be observed in the electron microscope, must be prepared to a thickness of less than 1 micron. However, since in the case of most materials it is very difficult with present day equipment to section or grind the material to such a thickness without undue distortion, an indirect method must be used to make surface

[1] Received for publication April 18, 1944.

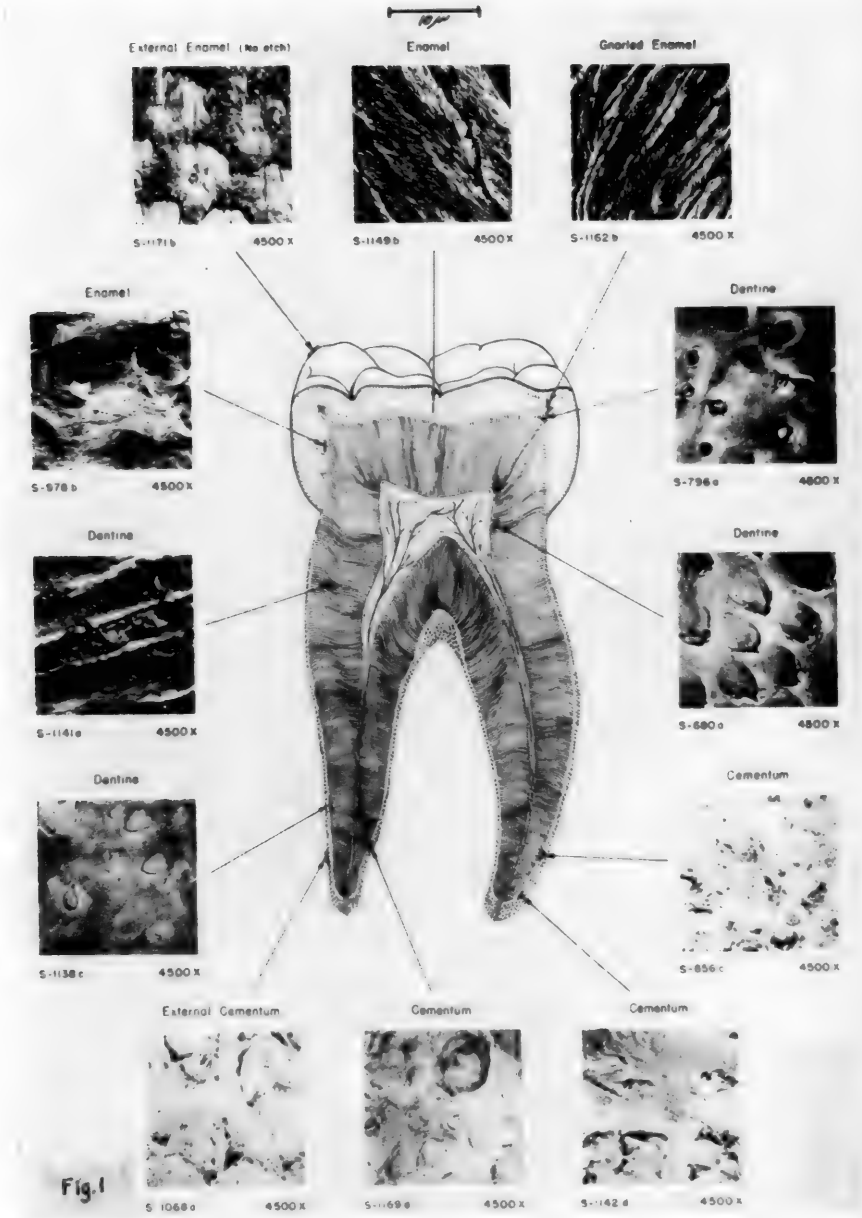


Fig. 1. Electron micrographs of etched surface and interior structures of a human tooth. Polystyrene-silica replicas.

studies. The polystyrene-silica replica technique quite adequately fills the necessary requirements. This technique lacks the simplicity of some others (2), but is more desirable because of the high quality of reproduction down to at least 0.01 microns (3).

POLYSTYRENE-SILICA REPLICA TECHNIQUE

After the sample to be studied has been given the desired surface preparation, a plastic impression is made of the surface. This first step is accomplished by

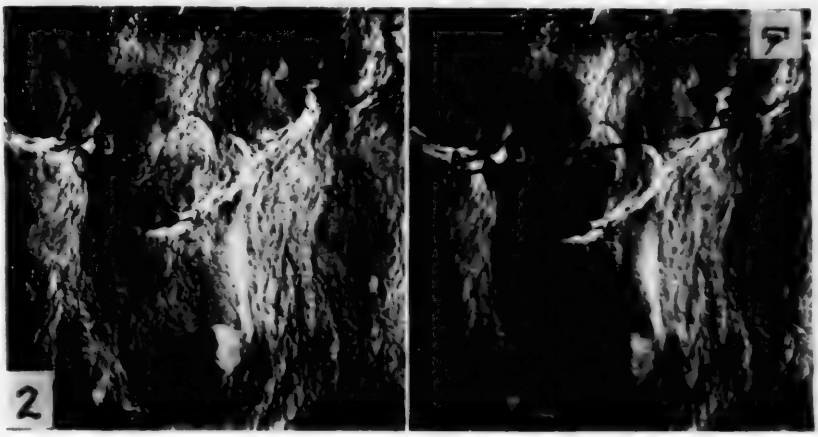


Fig. 2. Stereo electron micrograph of the etched enamel interface at the dentino-enamel junction ×3200.

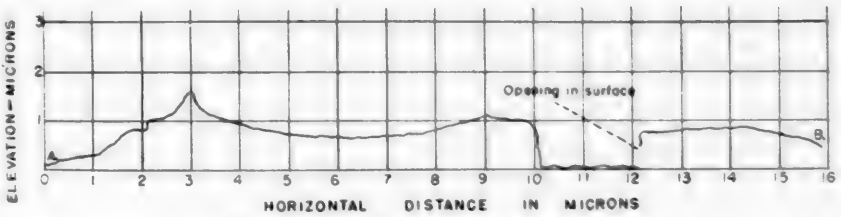


Fig. 3. Profile along line AB in *fig. 2*

molding polystyrene granules against the specimen surface at a temperature of 160°C. and a pressure of approximately 3000 pounds per square inch. After molding, the excess polystyrene is removed from the edges and the original specimen is either stripped off or dissolved in acid or caustic.

Silica is now evaporated on the styrene replica surface by means of a conical tungsten filament in vacuum. A relatively uniform silica film is obtained due to the high mobility of the condensing silica which allows silica molecules to migrate readily both on the polystyrene and on the silica that has already been deposited. The ideal thickness of silica film seems to be about 0.02 microns.

The silica film is cut into squares and removed from the polystyrene with ethyl bromide solvent, washed free from polystyrene and caught on specimen screens which are placed in the electron microscope.

The interpretation of electron micrographs (3) on the basis of intensity distribution of the image is encumbered with many difficulties and is quite inconclusive by itself. This is due to the fact that the intensity distribution is affected by a number of indefinite and varying factors such as the film thickness, height of projections, depth of pits, slope of film on walls of valleys and ridges, etc.

A much more positive method of interpreting electron micrographs is made available by the use of stereoscopy. Stereomicrographs, having a total angle between the 2 pictures of 8 degrees, can be readily obtained in the present electron microscope using silica replicas. When viewed through a stereoscope, such stereo-pairs are very striking in their third-dimensional quality, especially where abrupt elevation changes occur. *Fig. 2* shows a stereo-pair of the enamel interface at the dentino-enamel junction of a human molar. This surface was obtained by breaking the dentin and enamel apart and preparing a replica of the exposed enamel surface after light etching. When only one of the stereo pictures is examined visually, an impression of a rolling surface is obtained, but it is quite indefinite. However, the use of the stereoscope definitely shows the true surface contours. *Fig. 3* shows the profile or surface contour along the line AB of *fig. 2*, as determined by the contour finder, a quantitative stereoscopic instrument used in aerial mapping.[2]

PREPARATION OF TOOTH REPLICAS

The teeth were sectioned with a water-cooled, $\frac{1}{16}$ inch carborundum cutting wheel. Following sectioning the teeth were polished down through 4/0 metallographic paper and then etched. Dilute aqueous solutions of hydrochloric acid were used for etching, the concentration and etching time varying with the composition and solubility of the particular region being studied. For the readily soluble enamel a 0.2% hydrochloric acid solution with an etching time of 2 or 3 seconds was used. For the less soluble dentin and cementum a 1 to 2% hydrocholic acid solution with an etching time of about 5 seconds was used.

After a tooth had been molded against polystyrene and the excess styrene trimmed away, the tooth was separated from the styrene by mechanical stripping if possible. In most cases with dentin and cementum structures this stripping was readily accomplished. However, enamel structures generally resisted mechanical stripping unless the etching was very light. In such cases the tooth section was dissolved in 15% hydrochloric acid for enamel structures and 15% hydrochloric acid plus 5% hydrofluoric acid for dentin and cementum sometimes followed with 50% nitric acid in order to remove organic residues.

One of the difficulties experienced in this work was the recognizing of specific tooth regions in the electron microscope. This difficulty was overcome to a large extent by examining the polystyrene replica in the light microscope at magni-

[2] The application of this technique is described in an article in the Journal of Applied Physics (Heidenreich, R. D., and Matheson, L. A., Electron microscope determination of surface elevations and orientations, *J. Appl. Phys.*, **15**: 423, 1944).

Fig. 4. External enamel surface of upper crown of canine—not etched (orig. mag. ×5300).

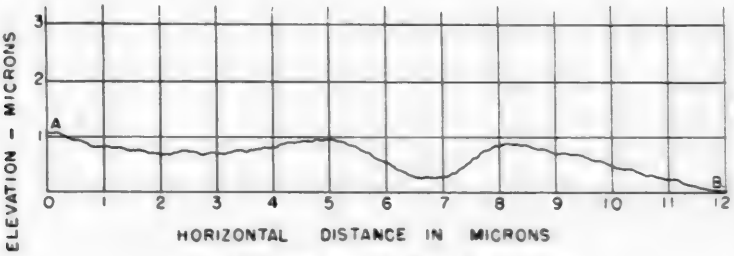


Fig. 5. Profile along line AB in *fig. 4*

Fig. 6

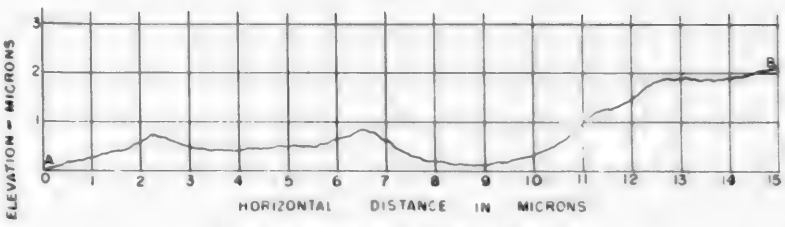
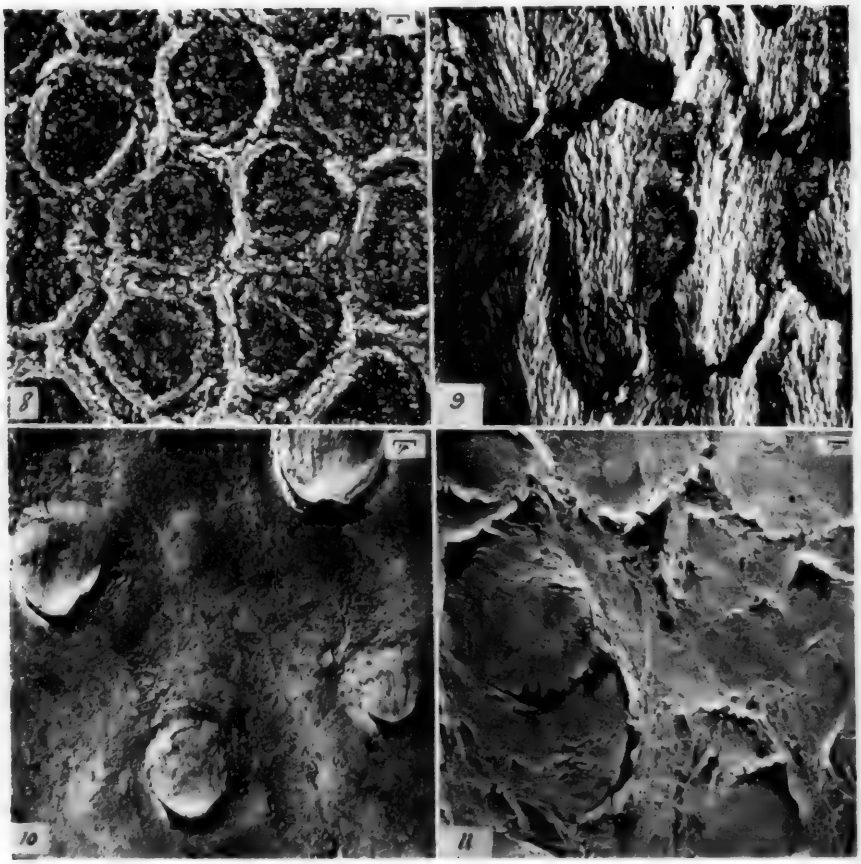


Fig. 7. Profile along line AB in *fig. 6*



Figs. 8–11. Enamel (*8, 9*), dentin (*10*) and cementum (*11*) surfaces

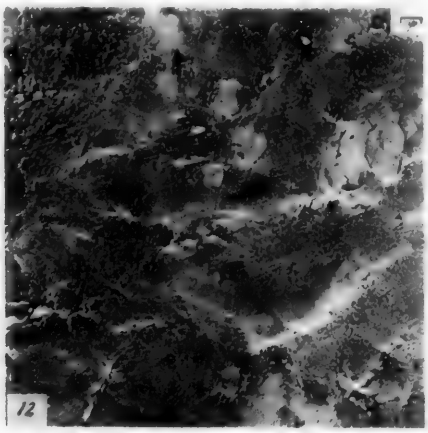


Fig. 12. Internal cementum of molar—etched (orig. mag. ×5300)

fications of 100 to 600 times, using illumination through the styrene. Thus the specific region of interest could be circumscribed with a stylus and silica film removed from only the circumscribed region. This film could then be examined in the electron microscope at magnifications as low as 1500 times and the regions quite readily recognized. Photographs may be taken at any magnification between 1500 and 15,000 times and then photographically enlarged as desired.

ELECTRON MICROGRAPHS OF TOOTH STRUCTURES

In the following section are presented a number of electron micrographs which in general are representative of tooth structures and show what can be accomplished with the electron microscope and the polystyrene-silica replica technique. These are as follows:

Fig. 4. External enamel surface of the upper crown of a canine—No etch. ×5300 orig. mag. *Fig. 5.* Profile along line AB in *fig. 4*. *Fig. 6.* Dentin from crown of canine. Dentinal tubules cut nearly longinal—Etched. ×5300 orig. mag. *Fig. 7.* Profile along line AB in *fig. 6*. *Fig. 8.* Enamel from incisor of dog—Etched. ×5300 orig. mag. *Fig. 9.* Enamel from human molar—Etched. ×5300 orig. mag. *Fig. 10.* Dentin from molar with dentinal tubules cut transversely—Etched. ×5300. *Fig. 11.* External cementum surface of canine—Etched. ×5300 orig. mag. *Fig. 12.* Internal cementum of molar—Etched. ×5300 orig. mag.

CONCLUSIONS

This preliminary investigation indicates that the electron microscope can be effectively applied to tooth structures using surface replicas. The success of this method will depend on the surface preparation bringing out the structural details as in the case of metallography.

The interpretation of the electron micrographs is greatly facilitated by the use of the stereoscopic technique which can be both qualitative and quantitative. It is hoped that advantage will be taken of the possibilities of further applying the electron microscope to dental research.

The author wishes to express his appreciation to Dr. D. A. Kerr and Lt. F. K. Bradley for their helpful discussion, suggestions, and criticism.

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DOMESTIC WATER AND DENTAL CARIES[1]

VI. The Relation of Fluoride Domestic Waters to Permanent Tooth Eruption

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Various observations (1, 2, 3) have been made indicating an apparent delay in tooth eruption in individuals living in areas of endemic dental fluorosis; other investigators (4, 5) have failed to confirm these findings. These observations, however, have dealt largely with areas of high endemicity, the concentration of fluorides in the drinking waters being relatively high. It has been reported (6) that the feeding of high concentrations of fluorides to rats has substantially reduced the rate of eruption of the lower incisor teeth. As a result of recent field studies concerning the effect of smaller concentrations of fluorides on dental caries (7, 8), certain data have been made available permitting the investigation of evidence concerning any delay in permanent tooth eruption in areas of lower fluoride concentration. Because of the inverse relationship between endemic dental fluorosis and dental caries prevalence, it seemed advisable to determine, if possible, whether this variable might disclose an appreciable difference in years of post eruptive tooth exposure to the risk of dental caries.

The 8 Chicago suburban communities (7) besides presenting unusual opportunities to study dental caries, also offer certain advantages in the study of comparative eruption rates. These advantages include homegeneity of populations studied, similarity of climatological factors, and range in fluoride (F) content of the public water supplies, 0.0–1.8 parts per million. All dental examinations were made by one of two examiners (Assistant Dental Surgeons (R) David C. Johnston and Edwin M. Short) with approximately an equal number in each city being done by each examiner. In addition to these 8 communities, Galesburg, Illinois, with a water supply containing approximately the same concentration of fluorine as Elmhurst, the highest fluoride community in the Chicago area study, is included in this series along with the control city of Quincy, which uses fluoride free Mississippi River water. In order to determine the effect, if any, on tooth eruption of a public water supply containing more fluorine than any of those studied in Illinois, Colorado Springs, Colorado, the water supply of which contains the highest concentration of fluorine of any city surveyed by the same examiners, is also included in this series of cities. In order to provide a measure of control against this high fluoride community, the nearby city of Pueblo, whose water supply is relatively low in fluoride content, is also included.

[1] Received for publication May 3, 1944.

[2] At present, on duty U. S. Marine Hospital, Stapleton, N. Y.

In this series of 12 cities, 4 (Quincy, Evanston, Oak Park, and Waukegan) have water supplies that are virtually fluoride free; 2 (Elgin and Pueblo) use water containing approximately 0.5 ppm. of fluoride (F); the water supplies of 3 communities (Joliet, Maywood, and Aurora) contain slightly over 1 ppm., and those of 2 more (Galesburg and Elmhurst) have just below 2 ppm. The highest fluoride city in this series, namely Colorado Springs, uses a water supply the fluoride (F) content of which is 2.6 ppm. An inverse relationship between fluoride content of the public water supply and dental caries prevalence rates has been demonstrated in these 12 communities (8); those cities having more than 1 ppm. of fluoride (F) in their water supplies show from one-half to one-third the caries experience rates of the fluoride free cities. Those 2 communities using water of approximately 0.5 ppm. of fluoride (F) lie approximately midway between the higher fluoride and fluoride free communities in caries experience rates.

On the basis of a previously described method of computing a community mottled enameled index (9), 3 of these 12 cities (Colorado Springs, Galesburg, and Elmhurst) would be classified as "slight" and 3 (Joliet, Maywood, and Aurora) as "borderline." The 6 remaining communities show a "negative" mottled enamel index. Those communities whose public water supplies contain fluorine in concentrations between 1 and 2 ppm. show a very mild type of dental fluorosis characterized by a few small white opaque areas on a small percentage of the permanent teeth.

In the dental examinations as conducted in the 4453 children, age 12 to 14 years,[3] of these 12 communities, definite information was recorded for each tooth, erupted or unerupted. Erupted teeth were marked as normal or carious, i.e., untreated, filled, missing, or indicated for extraction. Only those teeth that had erupted to the point of being in occlusion or in line of occlusion were classified as erupted. Unerupted teeth were marked as such. Intermediately is the partially erupted tooth, a tooth being placed in this classification from the time the point of the cusp makes it appearance through the mucosa until it may be classified as fully erupted.

DATA

Table I shows the mean number of erupted and partially erupted teeth in each of the 12 communities studied. They are placed in the order of decreasing fluoride content of the public water supply.

In Tables II, III, and IV the cities have been grouped according to the fluoride content of the public water supply. Group 1 consists of Colorado Springs, using a domestic water containing 2.6 ppm. of fluoride (F). Group 2, consisting of Galesburg and Elmhurst, use public water supplies of 1.9–1.8 ppm. fluoride (F) content. In group 3 are Joliet, Maywood, and Aurora, whose water supplies contain fluoride (F) in concentrations of 1.3–1.2 ppm. Group 4 is made up of Pueblo and Elgin with 0.6–0.5 ppm. of fluoride (F) in their drinking waters. Quincy, Evanston, Oak Park, and Waukegan make up group 5; the water sup-

[3] Age of child was determined on basis of age at last birthday.

plies of these cities have 0.1–0.0 ppm. of fluoride (F), a concentration which may be considered "fluoride-free." The distribution of the children by age and sex within each group is shown according to the total number of erupted permanent teeth.

TABLE I

Summary of permanent tooth eruption data, third molars excluded, of selected 12-14 year old white school children of 12 communities in Illinois and Colorado

CITY	NUMBER OF CHILDREN EXAMINED			ERUPTED PERMANENT TEETH* (a)	PARTIALLY ERUPTED PERMANENT TEETH (b)	TOTAL (a + b)
	M	F	Both			
				(A) Number		
Colorado Springs, Colo. (2.6)**	183	221	404	10190	235	10425
Galesburg, Ill. (1.9)	137	136	273	6987	162	7149
Elmhurst, Ill. (1.8)	89	81	170	4414	90	4504
Joliet, Ill. (1.3)	196	251	447	11984	132	12116
Maywood, Ill. (1.2)	92	79	171	4462	81	4543
Aurora, Ill. (1.2)	346	287	633	16559	253	16812
Pueblo, Colo. (0.6)	314	300	614	16088	259	16347
Elgin, Ill. (0.5)	199	204	403	10605	170	10775
Quincy, Ill. (0.1)	142	188	330	8725	146	8871
Evanston, Ill. (0.0)	111	145	256	6683	122	6805
Oak Park, Ill. (0.0)	138	171	329	8634	159	8793
Waukegan, Ill. (0.0)	190	233	423	11085	214	11299
				(B) Number per Child		
Colorado Springs, Colo.				25.22	0.58	25.80
Galesburg, Ill.				25.59	0.59	26.19
Elmhurst, Ill.				25.96	0.53	26.49
Joliet, Ill.				26.81	0.30	27.11
Maywood, Ill.				26.09	0.47	26.57
Aurora, Ill.				26.16	0.40	26.56
Pueblo, Colo.				26.20	0.42	26.62
Elgin, Ill.				26.32	0.42	26.74
Quincy, Ill.				26.44	0.44	26.88
Evanston, Ill.				26.11	0.48	26.58
Oak Park, Ill.				26.24	0.48	26.73
Waukegan, Ill.				26.21	0.51	26.71

* Permanent teeth which have been extracted are counted as erupted.

** Figure in parenthesis shows fluoride (F) content of public water supply in parts per million.

Table V is used as the basis of a comparison of the group of 0.0–0.1 ppm.(F) communities (No. 5) with each of the other groups (Nos. 1, 2, 3, and 4). The comparisons are made by single age for both sexes. The chi-square (X^2) values are computed for each of the comparisons made, permitting the determination

TABLE II

Distribution of 2150 white school boys, age 12–14 years, according to number of erupted permanent teeth. Children grouped on basis of fluoride (F) content of their public water supply

NO. OF ERUPTED PERMANENT TEETH	12 YEARS					13 YEARS					14 YEARS				
	2.6*	1.8–1.9	1.2–1.3	0.5–0.6	0.0–0.1	2.6	1.8–1.9	1.2–1.3	0.5–0.6	0.0–0.1	2.6	1.8–1.9	1.2–1.3	0.5–0.6	0.0–0.1
28	12	18	78	41	55	25	41	117	97	116	46	42	147	135	145
27	5	3	16	14	15	5	8	37	22	24	6	11	21	13	23
26	7	8	15	18	18	6	9	18	31	26	3	5	11	6	16
25	4	6	18	10	13	3	4	7	5	12	2	3	6	3	5
24	5	12	17	15	21	6	5	14	8	15	3	4	4	5	4
23	1	3	14	10	7	2	1	4	6	5		2	1	1	2
22	1	8	13	7	16		1	2	2	1			2	2	1
21	6	4	6	8	12	2	2	3	5	1			1	1	2
20	1	2	7	8	2	3	1	2	3	4			1	1	
19	2	4	7	11	6		1	4	1	1					
18	6		9	6	5		1	1	3	2					
17	3	2	5	5	2	1						2	1		
16	1	4	3	2	1		2	4	1	1					
15	3	2	4	1	3	1		1	2						
14		3	4		2	2		1		2					1
13	2	1	2	1	3			2							
12	8		3	1	3		1	1							
11				1											
10				1	1										
Total.....	67	80	221	160	185	56	77	218	186	210	60	69	195	167	199
Mean no. of erupted teeth per child	21.55	23.24	24.18	23.82	24.10	25.32	26.05	26.23	26.34	26.52	27.50	26.87	27.39	27.49	27.35

* Fluoride (F) content of public water supply in parts per million.

TABLE III

Distribution of 2303 white school girls, age 12–14 years, according to number of erupted permanent teeth. Children grouped on basis of fluoride (F) content of their public water supply

NUMBER OF ERUPTED PERMANENT TEETH	12 YEARS					13 YEARS					14 YEARS				
	2.6*	1.8–1.9	1.2–1.3	0.5–0.6	0.0–0.1	2.6	1.8–1.9	1.2–1.3	0.5–0.6	0.0–0.1	2.6	1.8–1.9	1.2–1.3	0.5–0.6	0.0–0.1
28	20	27	76	83	83	48	44	164	102	161	45	49	180	117	154
27	5	5	19	21	42	14	9	26	23	42	8	4	18	15	37
26	10	6	12	22	23	7	9	13	16	23	8	3	12	2	16
25	1	4	15	16	19	5	2	7	8	17	2	3	6	1	7
24	9	15	11	13	21	1	8	5	3	10			4	2	5
23	6	4	5	7	12	1	4	4	5	3	1	1		2	2
22	1	3	8	11	12		1	4	7	8			1	1	1
21	2	1	6	7	9	2	1	2	2	4					1
20	3	2	5	1	4	1	2		2	2					
19	4	2	5	3	3							1			
18	1	3	1	3	5				2	2					
17	5	1	2	2	5	1	1		2				1		
16	1				2	1		2	2						
15					5		1								
14			2		2		1								1
13	1		1	1											
12	7				1										
Total.....	76	73	168	190	248	81	83	227	174	272	64	61	222	140	224
Mean no. of erupted teeth per child	23.03	25.14	25.58	25.81	25.09	26.80	26.34	27.23	26.54	26.88	27.45	27.46	27.58	27.49	27.34

* Fluoride (F) Content of public water supply in parts per million.

TABLE IV

Distribution of 4453 white school children, age 12–14 years, according to number of erupted permanent teeth

NUMBER OF ERUPTED PERMANENT TEETH	I 2.6 PPM. F		II 1.8–1.9 PPM. F		III 1.2–1.3 PPM. F		IV 0.5–0.6 PPM. F		V 0.0–0.1 PPM. F	
	M	F	M	F	M	F	M	F	M	F
28	83	113	101	120	342	420	273	302	316	398
27	16	27	22	18	74	63	49	59	62	121
26	16	25	22	18	44	37	55	40	60	62
25	9	8	13	9	31	28	18	25	30	43
24	14	10	21	23	35	20	28	18	40	36
23	3	8	6	9	19	9	17	14	14	17
22	1	1	9	4	17	13	11	19	18	21
21	8	4	6	2	10	8	14	9	15	14
20	4	4	3	4	10	5	12	3	6	6
19	2	4	5	3	11	5	12	3	7	3
18	6	1	1	3	10	1	9	5	7	7
17	4	6	4	2	6	3	5	4	2	5
16	1	2	6		7	2	3	2	2	2
15	4		2	1	5		3		3	5
14	2		3	1	5	2			5	3
13	2	1	1		4	1	1	1	3	
12	8	7	1		4		1		3	1
11							1			
10							1		1	
Total....	183	221	226	217	634	617	513	504	594	744
Mean no. of erupted teeth per child	24.66	25.69	25.31	26.18	25.87	26.91	25.93	26.58	26.04	26.42

TABLE V

Comparison of 5 groups of children, age 12 to 14 years, classified on basis of fluoride content of public water supply, showing chi-square values and probability of results occurring by chance

GROUPS COMPARED**	VALUE OF X^2 AND P ACCORDING TO AGE AND SEX OF CHILDREN*											
	12 Years				13 Years				14 Years			
	Males		Females		Males		Females		Males		Females	
	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P
1 vs. 5	16.84	0.0021	13.26	0.0042	7.81	0.0698	0.39	0.8465	0.34	0.5598	0.06	0.8065
2 vs. 5	4.07	0.3977	3.56	0.3187	3.04	0.3864	2.95	0.2304	3.50	0.0614	3.14	0.7843
3 vs. 5	2.23	0.6950	1.65	0.6525	3.94	0.2693	4.10	0.1300	0.33	0.5657	9.02	0.0027
4 vs. 5	5.02	0.2855	4.06	0.2561	3.71	0.2992	2.57	0.2854	3.21	0.0736	9.95	0.0016

* For purposes of calculating X^2 the following values of N^1 were used: 12 Years Males $N^1 = 5$, females $N^1 = 4$; 13 Years Males $N^1 = 4$, females $N^1 = 3$; 14 Years Males $N^1 = 2$, females $N^1 = 2$.

** Group 1 = 2.6 ppm. fluoride (F); group 2 = 1.8 − 1.9 ppm; group 3 = 1.2 − 1.3 ppm; group 4 = 0.5 − 0.6 ppm.; group 5 = 0.0 − 0.1 ppm.

of P. which represents the probability that the differences observed are likely to be due to chance.

DISCUSSION

When the children of Colorado Springs, or group 1 (Table V) are compared with group 5, composed of children from cities using fluoride free waters, a significant difference is disclosed in the eruption rates of the permanent teeth for the 12 year old age group of both sexes Thus, children 12 years of age in the higher fluoride cities show an appreciably lower eruption rate than those in the fluoride free group.[4] A similar condition prevails to a lesser degree in the 13 year old boys but the 14 year olds, both sexes, show no significant difference.

Group 2 consisting of the next highest fluoride (F) cities (1.8–1.9) ppm. in this series, shows no significant difference from group 5 in eruption rates, although the 12 and 13 year old boys of group 2 are slightly behind the boys of the same age in group 5. The next 2 groups of cities, Nos. 3 and 4, using water supplies with fluoride (F) contents of 1.2–1.3 and 0.5–0.6 ppm., respectively, show no evidence of a significant delay in eruption at any of the ages examined; in fact, at some ages in each group there is a tendency toward the opposite effect of a slightly higher eruption rate.

The basic purpose in analyzing the data from these population groups was to determine whether or not appreciable difference existed in the time of eruption of the permanent teeth. If for instance, significant differences were found to exist between those characterized by low dental caries prevailence in contrast to those having a high dental caries experience, the influence of this time variable respecting risk of exposure to dental caries attack would necessitate careful appraisal. But, as has been shown above (Tables II, III, and IV), the marked differences in dental caries experience (8) between populations using fluoride-free domestic waters and those using waters containing as much as 1.2–1.3 parts per million of fluoride (F) cannot be explained on the basis of differences in the time of eruption.

The disclosure of a significant difference in the eruption rate recorded for the Colorado Springs population (2.6 ppm. of F in the public water supply) seemingly warrants further comment in the light of its possible relation to other closely related physiological fields.[5] Acceleration or retardation of permanent

[4] It should be pointed out that in group I, the distribution of 12 yr. old children according to the number of erupted permanent teeth shows an unusually large number with 12 erupted teeth. When all children with 12 or less erupted teeth are eliminated from both groups, a comparison of the 12 year olds of group I with those in group V shows a significant difference for the boys ($P = 0.0445$) but not for the girls ($P = 0.2050$).

[5] The unusual effects of trace elements on physiological processes has opened a vast field for scientific investigation. Moulton (11) referring to dental fluorosis has recently stated that "It is a new and quite different illustration of the fact that very minute quantities of certain elements and of certain organic compounds (vitamins and hormones) sometime have extraordinary effects upon living organisms." More recently Shils and McCollum (12) state that like vitamins, the indispensable "traces" appear to be keys to fundamental physiologic processes little understood and a challenge to nutritionists and physiologists. The investigation of the physiological effects of one trace element, fluoride, by the epide-

tooth eruption is a matter of interest to both anthropoligists and those concerned with growth and development. Racial differences in eruption time have been reported by Steggerda and Hill (10), who state that in the 4 racial groups studied, the early eruption time of teeth to be in the following order: Navajo, Negro, Maya, and White. In the data reported in the present paper the genetic constitution and environmental conditions (fluoride intake excluded) would seem largely comparable in the 12 white school populations studied in Illinois and Colorado. The only variable recognizable at present which may account for the significant difference noted at Colorado Springs would seem the higher fluoride intake. The suggestion that one growth index (rate of permanent tooth eruption) might be influenced by a minute fluoride intake raises the question of the possible influence of this variable in studies, other than dental, in closely coordinative physiological fields.

Although it is possible that the difference in eruption between the high fluoride and fluoride-free cities may be due to the lower dental caries experience rates in the high fluoride areas resulting in a longer retention of the deciduous teeth, this seems improbable, as the dental caries prevalence rates in these high fluoride areas are comparable to those found in the more moderate fluoride areas which show no lower eruption rates than the fluoride-free cities.

SUMMARY

1. Public water supplies containing fluorides in concentrations of 2.6 parts per million appear to be associated with delayed permanent tooth eruption in children 12 years of age who have been continuously exposed to the water supply. Significant differences were not observed in those populations using waters containing less than 2.0 parts per million of fluoride (F).

2. The marked difference in dental caries prevalence between populations using fluoride-free domestic waters and those using as much as 1.2–1.3 parts per million of fluorides (F) cannot be explained on the basis of differences in time of permanent tooth eruption.

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INTRA-ORAL HYDROGEN-ION CONCENTRATIONS ASSOCIATED WITH DENTAL CARIES ACTIVITY[1]

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The chemico-parasitic theory of the etiology of dental caries which was advanced by W. D. Miller in 1890 (1) postulates that the decalcification of tooth substance is caused by acids, and that these acids are produced from carbohydrate foods by bacteria growing in the mouth. This theory is more or less generally accepted today, although the possibility exists that agents other than acids may effect decalcification of teeth in caries.[2]

The chief criticism of the chemico-parasitic theory has been that it fails to explain the facts known about "susceptibility" and "immunity" to caries. For example, it has been pointed out that acid producing bacteria grow on the teeth of individuals who are free of caries as well as those who have caries (9). A common objection to the theory has been that the ingestion of carbohydrates in the diet does not necessarily lead to the development of caries. Nevertheless, these objections would be invalidated if it could be shown that the bacteria and carbohydrates produce a significantly greater acidity on the teeth of "caries-susceptible" individuals than on the teeth of "caries-immune" people.

Until recently very little was known about the hydrogen-ion concentrations which develop on the teeth. The only direct evidence which Miller (1) was able to offer in order to show that acidity occurs in caries, was the demonstration that blue litmus paper generally turns red when pressed against carious dentin. Later, when methods were developed for the determination of acidity in terms of hydrogen-ion concentrations, there were many attempts to find a pH in the saliva which would explain caries. None have been successful. Most investigators have found the pH of saliva to be from 6.0 to 7.4 (10) although values as low as 5.3 have been reported (11). Hanke (12) in 1931 attempted to correlate the local acidity of different areas of the mouth with caries, but found no pH value below 5.4. Since evidence has accumulated that decalcification of teeth prob-

[1] The expenses of this investigation were defrayed in part by a grant from the Squibb Institute for Medical Research. Received for publication May 11, 1944.

[2] Examples of other possible mechanisms for decalcification are removal of phosphate ions by phosphorylation (2) and removal of calcium ions by complex ion formation with polycarboxylic acids (3, 4). Individual differences in calcium and phosphate concentrations of oral fluids would exert some effect on enamel solubility. Differences may exist in the solubility of enamel in different teeth (5). This may be influenced by the fluorine content of the enamel (6, 7). Also the enamel in a single tooth may not be uniform, as shown by differences in the surface and deeper portions of enamel (5, 8). In this regard it is well to remember that experiments done on powdered enamel do not necessarily give a true picture of conditions for solubility of the enamel surface of intact teeth.

ably occurs only at a pH of about 5.0 or below under mouth conditions[3] it is clear that none of these studies could explain on the basis of pH the decalcification which occurs in caries.

Etherington and Trimble (16) in 1934 reported pH readings of plaques as low as 4.6 by a macro-colorimetric test which apparently involved an excessive dilution factor, but the details of this method were not published. They found no differences between caries-active and caries-inactive individuals.

In 1938 Stephan (17) reported a study of the hydrogen-ion concentration of carious material and plaques, in which a suitable micro-colorimetric method for determination of pH was employed. He found pH values as low as 4.2. The average pH of carious material was .7 pH unit lower than that of plaques from intact tooth surfaces. In one mouth pH values of 5.0 and below were observed on an intact tooth surface which became carious during the period of study, whereas in the same mouth no pH of 5.0 or below was found on the tooth surfaces which remained intact throughout the period of study. It was also reported that the acidity of plaques was increased by the eating of candies and cookies.[4] In a later report (18, 19) in which the antimony electrode was employed for direct pH measurements in the mouth, it was shown that the ingestion of glucose, fructose, sucrose, maltose, or cooked starch produces a rapid fall in the pH of plaques and carious lesions, and that lactose produces a relatively small drop in pH. Fosdick, Campaigne, and Fancher (20) and Fosdick and Burrill (21), questioning the accuracy of the antimony electrode under the conditions of the mouth, "duplicated" these experiments by removing material from the teeth and testing for pH with a micro-glass electrode. They obtained similar results. However, they failed to find any drop in the pH of material removed from the teeth of "caries-immune" individuals after the ingestion of carbohydrates. Since acid-forming bacteria grow in the mouths of "caries-immune" individuals as well as in the mouths of "caries-susceptibles" (9), it seems important to establish the extent to which the pH on the teeth of these individuals may differ. The following study was made in order to determine the relationship existing between intra-oral hydrogen-ion concentrations and dental caries activity.

METHODS

Determination of Caries Activity. The caries activity of the individuals studied in this report was determined by a comparison of clinical and roentgenological examinations made over a period of from 1 to 2 years. The subjects were divided

[3] Holt, LaMer, and Chown (13) showed that when .01 M phosphoric acid is titrated with a saturated solution of calcium hydroxide, the pH remains at about 5.0 during neutralization of the second and third hydrogen ions from the phosphoric acid molecule. This is due to the precipitation of hydroxyapatite at this pH level. Enright, Friesell, and Trescher (14) showed that intact enamel was not dissolved above a pH of 5.0 in buffer solutions which had been previously saturated with calcium phosphate and then acidified to their original pH. Bibby, Volker, and Van Kesteren (15) found that the pH of cultures of oral bacteria did not fall below 5.0 in the presence of powdered enamel, and concluded that the enamel was dissolved at this pH.

[4] Paper read to The Odontographic Society of Chicago, April 11, 1938.

into 5 groups on the basis of the occurrence of new carious lesions, the increase in size of lesions which were present at the start of the study, and evidence of previous caries activity. Group I consisted of 5 individuals who were free from caries and had no evidence of any previous caries activity. Group II consisted of 11 individuals who had evidence of caries which occurred previous to the start of the study but who showed no new lesions, and no progress in old ones during the period of study. Group III consisted of 26 individuals who showed evidence of a slight but definite increase in the size of 1 or more carious lesions present at the start of the study period, and/or possibly the appearance of some very small new lesions, but in none of these cases was the progress of caries rapid. Group IV consisted of 15 individuals who showed a very definite progress in caries, with relatively rapid increases in the size of lesions present at the start of the study and the development of new carious lesions. Group V consisted of 8 individuals with extremely active caries. These individuals showed extensive carious lesions on most of the teeth, and progress of caries was very rapid in them. All but 1 had active caries on the labial surfaces of the anterior teeth, whereas only 2 individuals in group IV and none in the other groups had this type of caries. This division of the 65 individuals is somewhat arbitrary, but it is felt that in no case would the individual be misplaced more than 1 position from this correct relative caries-activity grouping.

Preparation for pH tests. The subjects were instructed not to brush their teeth for from 3 to 4 days before the pH determinations were to be made. This was for the purpose of securing a representative oral bacterial flora on the accessible tooth surfaces. They were requested not to eat sweets on the days of the tests, and the tests were run 1 or more hours after mealtime.

Procedure for pH tests. The subjects were seated in a semi-reclining position in a chair equipped with a headrest. They were instructed not to talk during the test. The labial surfaces of the upper and lower anterior teeth were generally chosen for the tests because of their accessibility. In some of the caries-active individuals, particularly those in Group V, the enamel was etched or cavities existed under the plaques on these tooth surfaces. Direct pH determinations were made on the tooth surfaces with a polished-stick antimony electrode using a vacuum tube potentiometer (Leeds Northrup Model 7662) according to a method previously described (18, 22). Measurements were also made of the pH on the gingival tissue, the cheeks near the parotid ducts, the floor of the mouth near the submaxillary ducts, and the dorsum of the tongue. The mouth was rinsed with 25 cc. of a 10% aqueous solution of glucose for 2 minutes following these initial pH determinations. The pH determinations were repeated 2 minutes after the glucose rinse and at 10 minute intervals thereafter until the pH had returned to approximately its original value.

RESULTS

Before the application of glucose, the pH values of most areas were around neutrality except in the cases of extreme caries activity, in which pH values were generally considerably lower. In all cases there was a sharp drop in the pH of

bacterial material on teeth following rinsing the mouth with the glucose solution. This drop in pH was greatest and lasted for the longest time in the caries-active cases.

Fig. 1 shows the pH changes produced on the labial surfaces of the upper teeth for each caries-activity group. *Fig. 2* shows similar changes for the labial surfaces of the lower teeth. These graphs show that there are striking differences between the different caries-activity groups. However, there was no essential difference between those who were completely caries-free (Group I) and those

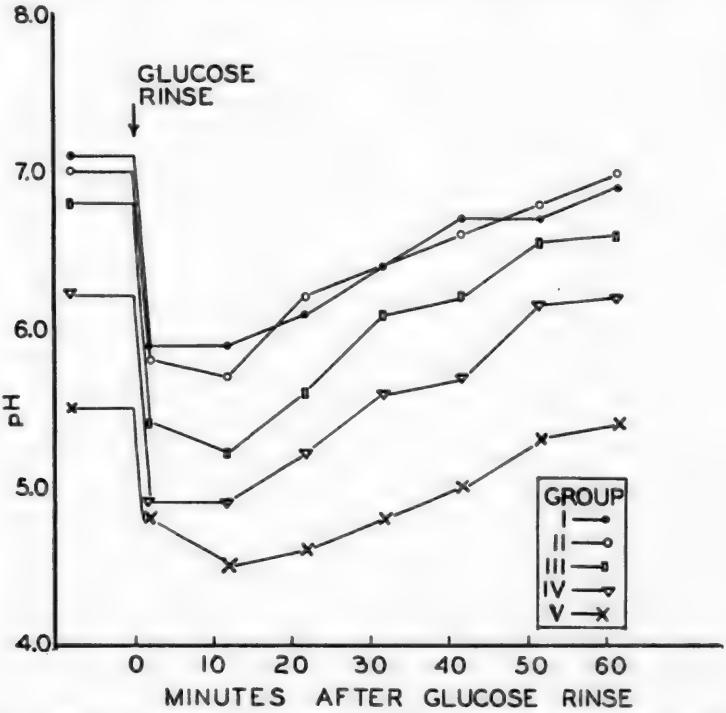

Fig. 1. pH Curves of plaques on labial surfaces of *upper* anterior teeth in different caries activity groups. Groups: I = caries-free; II = previous caries but caries-inactive during period of study; III = slight caries-activity; IV = marked caries-activity; V = extreme caries-activity.

who had had previous caries but were caries-inactive during the period of study (Group II). It is interesting to note that the pH drop in the lower teeth was less than on the upper teeth, and that this difference between the lower and upper teeth was smallest in the extremely caries-active cases.

Table I lists comparative pH values for the individuals in each of the caries-activity groups. The figures listed under *range* cover the variations of individual cases within each group and the figures listed under *average* summarize each group. The pH values for the saliva (measured on the floor of the mouth) show

a trend toward acidity in the caries active groups, although there is an overlapping of individual cases in all groups. In no case could acidity of this saliva itself account for decalcification of teeth. The pH values on tooth surfaces before application of glucose (initial pH) show an increase in acidity with increased caries activity. The range (pH 6.4 to 8.3) of the caries-inactive cases (Groups I and II) is completely above the range (pH 5.1 to 6.3) of the extremely caries-

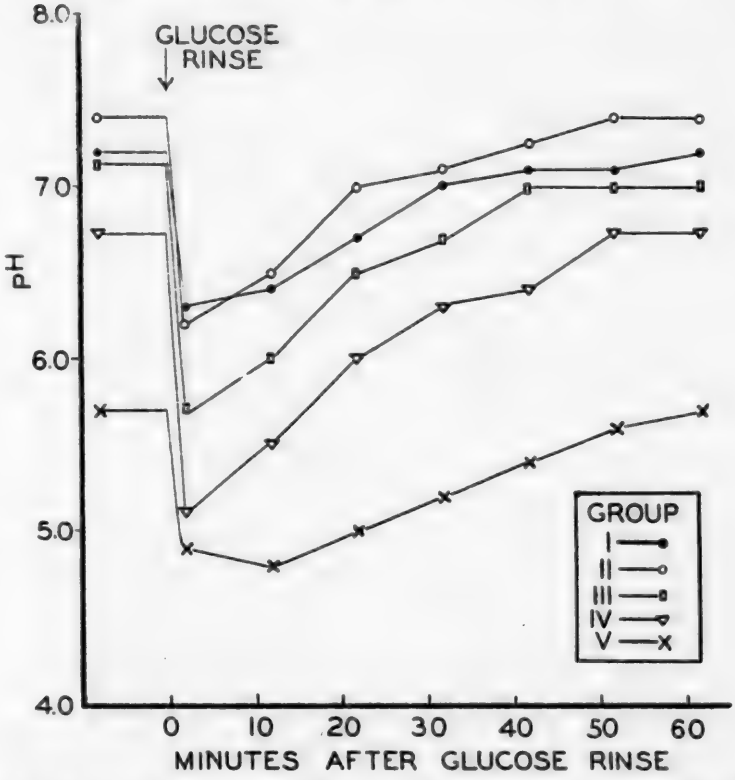

Fig. 2. pH curves of plaques on labial surfaces of *lower* anterior teeth in different caries activity groups. Groups: I = caries-free; II = previous caries but caries-inactive during period of study; III = slight caries-activity; IV = marked caries-activity; V = extreme caries-activity.

active cases (Group V). However, in none of these cases was a pH of 5.0 or below found on accessible tooth surfaces before the application of carbohydrate.

Probably the most interesting contrast found between the different caries-activity groups is in the comparison of the lowest pH values which occurred in each case after the application of glucose. Only in the caries-active cases were pH values below 5.0 consistently produced by the glucose.

Perhaps the most significant way to relate the pH changes to the caries-activity of the different groups is to classify the values according to a "hypothetical

TABLE I

Differences in pH values for different caries-activity groups

CARIES-ACTIVITY GROUP	NUMBER OF CASES	pH OF SALIVA (MEASURED ON THE FLOOR OF THE MOUTH)		INITIAL pH OF PLAQUES (BEFORE GLUCOSE)		LOWEST pH OF PLAQUES (AFTER GLUCOSE)	
		ave.	range	ave.	range	ave.	range
I (caries-free)	5	7.0	6.8–7.4	7.1	6.4–7.7*	5.5	5.2–5.8*
				7.2	6.7–7.5x	6.1	5.6–6.7x
II (caries-inactive)	11	7.0	6.2–7.3	7.0	6.6–7.5*	5.4	5.1–6.0*
				7.4	6.8–8.3x	5.9	5.7–6.4x
III (slight caries-activity)	26	6.7	6.0–7.0	6.8	6.2–7.7*	4.9	4.6–5.2*
				7.2	6.6–7.6x	5.4	4.7–6.5x
IV (marked caries-activity)	15	6.5	5.8–7.1	6.2	5.8–7.0*	4.6	4.3–4.8*
				6.7	6.2–7.4x	5.0	4.5–6.5x
V (extreme caries-activity)	8	6.4	5.9–7.0	5.5	5.1–6.3*	4.3	4.0–4.7*
				5.7	5.5–6.1x	4.8	4.0–5.2x

* Labial surfaces of the upper anterior teeth.
x Labial surfaces of the lower anterior teeth.

TABLE II

Frequency of Low pH Values in Plaques After Application of Glucose for Different Caries-Activity Groups

CARIES ACTIVITY GROUP	NUMBER OF pH DETERMINATIONS COMPARED	DETERMINATIONS AT OR BELOW pH 5.0		DETERMINATIONS AT OR BELOW pH 5.5	
		number	per cent	number	per cent
I (caries-free)					
upper	105*	0	0	7	7
lower	66x	0	0	0	0
II (caries-inactive)	219*	0	0	59	27
	93x	0	0	0	0
III (slight caries-activity)	483*	68	14	308	64
	219x	1	0	37	18
IV (marked caries-activity)	288*	168	58	279	97
	201x	35	17	83	41
V (extreme caries-activity)	167*	139	83	161	96
	73x	45	62	64	89

* Labial surfaces of upper anterior teeth.
x Labial surfaces of lower anterior teeth.

critical decalcifying pH level" above which calcium salts would not be dissolved from teeth under mouth conditions and below which decalcification would take place. While such a pH level has not been established exactly, and might vary in different invididuals, there is considerable evidence to indicate that pH 5.0 most nearly represents this value, and that a pH of about 5.5 probably represents the upper limit (see footnote 3). The number of times which pH values as low as these were reached in the first 30 minutes following application of glucose in a comparable series of pH determinations for each caries activity group are listed in Table II. This table shows that the extent of the caries activity is directly related to occurrence of pH values below such a critical limit. It also shows that bacterial material on the labial surfaces of the upper teeth in general developed a lower pH than that on the labial surfaces of the lower anterior teeth.

The pH of saliva measured on the floor of the mouth was not generally changed by the glucose rinse, but in some cases there were minor fluctuations. The pH of the cheeks near the parotid duct opening showed considerable variation between the pH value of parotid saliva and that of the plaques on the teeth against which the cheek rested. Apparently the parotid salivary secretion came in spurts which produced an irregular neutralization on the cheek mucosa of the acids which had diffused from the plaques on adjacent teeth. This variation was greatest in the cases with active caries.

An observation of interest is that there is a sharp drop in the pH on the dorsum of the tongue following the glucose rinse. This pH change is similar to that on the tooth surfaces. Apparently there are sufficient bacteria on the dorsum of the tongue to produce acid rapidly.

DISCUSSION

The results of this study indicate that there is a quantitative difference in the intensity and duration of the acidity produced by carbohydrates on the teeth of caries-free and caries-active individuals. This difference seems to be capable of explaining on a chemical solubility basis, the fact that decalcification of teeth with cavity formation does not occur in the former individuals but does in the latter. Nevertheless, the findings do not exclude the possible importance of factors other than pH which might produce or modify the decalcification of teeth in caries (see footnote 2).

Since most of the work which has been reported on acid production by mouth bacteria has not simulated conditions of the mouth, it is worth pointing out some essential differences between such studies and the experiments described here. The bacteria growing on tooth surfaces exist in concentrated masses or "plaques" which possess a maximum concentration of enzymes and coenzymes in a minimum space with minimum buffering capacity. The acid production by such bacterial masses is very rapid when carbohydrate is available and, since little initial buffering capacity must be overcome, the drop in pH is likewise very rapid. On the other hand, in experiments in which saliva is mixed with carbohydrates or culture media containing carbohydrates is innoculated with mouth microorganisms the pH drop during incubation is relatively slow because the concen-

tration of bacteria is small and the total buffer capacity great. One is not justified in assuming that the pH produced by incubating such mixtures for a longer time will be the same as the pH produced in a shorter time in the mouth, since dilution does not effect various enzymatic processes uniformly (4).

The question arises as to what factors are responsible for the pH differences in the different degrees of caries activity. There are many factors which may exert some effect to either increase or decrease the pH on tooth surfaces. Among these are (a) the buffering capacity of saliva, (b) the rate of flow of saliva, (c) the quantity of bacterial material on the teeth, (d) the type of bacteria on teeth and their ability to produce acid, (e) the ability of the bacteria to consume the acid produced, (f) the ability of the bacteria to produce alkaline substances, (g) the quantity of fermentable carbohydrate in the diet, and (h) the retention of carbohydrates in the mouth. Some of these factors have been correlated with caries-activity in studies which are well known. The relative importance of these and other factors is not very clear at the present state of our knowledge. It seems probable that a combination of such factors must govern the developments of caries in any given case.

What have the experiments described in this paper indicated in regard to the relative influence of these factors? Three things appear significant. First, bacterial plaques which are capable of producing acid rapidly grow not only on the teeth of individuals who have active caries, but also on the teeth of those who are caries-free. However, since a greater hydrogen-ion concentration is generally produced in the plaques of caries-active mouths, it seems probable that these plaques differ in some way from the plaques in caries-free mouths. Evidence in support of this hypothesis has been furnished by the work of Blayney, Kesel and Wach (23) who found a difference between the morphology of bacteria in plaques from caries-active tooth surfaces and the morphology of bacteria in plaques from caries-free tooth surfaces. Also the studies of Bunting and Jay (24) have indicated a relation of oral lactobacilli to caries.

Second, the presence of carbohydrates *in the mouth* appears to be essential for the production of an acidity capable of decalcifying teeth. In these experiments, a pH below 5.1 was found only after the application of glucose. This should not be interpreted to mean that enamel will be dissolved appreciably by the momentary existence of a pH of 5.0 or below on the tooth surface. As a matter of fact, carious lesions have not formed on many of the tooth surfaces which developed pH values below 5.1 for a short time after the application of glucose. It is probable that a significant decalcification of tooth substance will be produced by carbohydrates only when they are frequently applied to the tooth surface or mechanically retained on it for much longer than a few minutes time.[5]

[5] It should be remembered that only the tooth surfaces and cavities which were readily accessible to the electrode have been studied in these experiments. Conditions in less accessible tooth surfaces and cavities may be somewhat different. For example, in a previous study (18) several interproximal carious lesions which had extensive caries in the dentin but only a small opening in the enamel were opened by operative means with a rubber dam in place to keep out the saliva. The carious dentin thus made accessible to the elec-

Third, the accessibility of tooth surfaces to a flow of saliva appears to be an important factor from these studies. The labial surfaces of the upper anterior teeth, which have relatively little access to saliva show a greater drop in pH than the labial surfaces of the lower teeth, which have a relatively great access to saliva. This greater pH drop in upper anterior teeth than lowers agrees well with the greater susceptibility of upper anterior teeth to caries than the lower anterior teeth. (In the cases studied, there were over 3 times as many carious lesions in the upper anterior teeth than there were in the lower anterior teeth.) Thus, not only does the correlation of pH with caries activity hold for different individuals, but also for different areas of the mouth in the same individual.

SUMMARY

In this study of 65 individuals, it was found that there was an increase in the acidity of bacterial material on the teeth in every case after the mouth was rinsed with a glucose solution. However, the extent and duration of this increase in acidity (drop in pH) varied with the degree of caries-activity, being greatest in cases with extensive caries-activity. An acidity below pH 5.0 occurred only in cases with some caries-activity, and the occurrence of these low pH values was most frequent in cases of greatest caries-activity. The pH drop was more marked on the labial surfaces of the upper teeth than on the labial surfaces of the lower teeth, and this difference corresponded with the relative susceptibility to caries of these tooth surfaces. The significance of these findings to the etiology of caries is discussed.

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trode had a pH below 5.0 many hours after the subjects had eaten any carbohydrates or other food. Presumably, either particles of carbohydrate foods were retained in these areas for a longer time than on the more accessible tooth surfaces, or else the saliva did not have sufficient access to neutralize the acid as quickly as in more accessible cavities (or both). This difference in the duration of acidity could account for the extensive undermining of the enamel which occurs in interproximal and occlusal caries but does not generally occur in labial, buccal, and lingual caries. It could also account for the arrest of caries which frequently occurs when occlusal and interproximal cavities are opened to ready access of saliva, as shown by Anderson (25).

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A COLORIMETRIC METHOD FOR THE DETERMINATION OF AMYLOLYSIS OF SALIVA[1]

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The activity of saliva in the digestion of starch has been investigated by many workers. Day (1) used the principle expounded by Wohlgemuth (8) for studying enzymatic activity. In a series of test tubes Day placed different amounts of saliva and added 5 ml. of 1% corn starch solution to each tube which was promptly placed in ice water until the series was completed. The tubes were incubated at 38°C for 20 minutes and returned to the ice water to inhibit digestion. Distilled water was added until the level was 1 inch from the top of the tube and 1 drop of N/10 iodine solution was added to each tube which was shaken at that time. The solutions in the tubes assumed various colors, depending upon the completeness of the digestion. Amylolytic activity was expressed in terms of the color or of the amount of digestion of 1.0 ml. of such a solution or some multiple which was a whole number, under the conditions of time and temperature employed.

Myers and Dellenbaugh (6) developed an involved colorimetric procedure that gave the amylolytic activity of saliva in terms of the percentage of starch converted to reducing sugars. Gore (2) used a method developed "along lines suggested by Mathews" (3). This was a spot-plate method. The amylolytic index was the time required, in minutes, for digestion to reach the end point. A yellow color was considered the end point.

The work of Myers and Adams (5) indicated that high chloride and to a lesser extent high phosphate content of the saliva increased the amylolytic activity of ptyalin. This group and also Day (1) found marked variations in amylolytic activity of saliva when comparing various individuals. Gore (2) tested 1 individual and found marked variations in amylolytic activity of saliva during 24 hour periods. Meyer, Golden, Steiner and Necheles (4) found that the pytalin activity of saliva from old people was much less than that of young adults.

The present problem was to devise a new method for the determination of amylolysis by saliva and to collect preliminary data on the variations in pytalin activity between different individuals and at various periods of the day.

MATERIALS AND METHODS

The method in this paper was based upon the use of a Klett-Summerson photoelectric colorimeter. The data were expressed in mg. of starch digested in 1 minute by 0.1 ml. of fresh, unstimulated saliva at room temperature.

Merck's soluble, reagent starch was placed in a calcium chloride dehydrator

[1] Received for publication May 11, 1944.

in a partial vacuum. After allowing several days for the water content to reach an equilibrium, the starch was carefully weighed on an analytical balance and added to distilled water. Approximately 0.175 gm. of starch was added to about 50 ml. of water. This was brought to 92°C in a water bath at which time the solution was clear and without lumps. The starch solution was diluted in a liter volumetric flask at room temperature so that 40 ml. of the solution contained 3.5 mg. of starch.

Exactly 0.05 N iodine solution was prepared and standardized according to the method described on page 752 of the United States Pharmacopoeia, 12th Revision (7). The iodine is added to the starch after digestion by the enzyme to develop a blue color the intensity of which was measured.

The colorimeter was equipped with a KS-54 filter. The instrument was switched on 5 minutes before using to permit it to reach a heat equilibrium. A fresh batch of starch solution was made up daily and standard readings were made before and after each group of tests. The amount of starch in the solution cell was arbitrarily decided by experimentally determining the concentration required to obtain readings of the standard near the upper limit of the most sensitive range of the colorimeter scale.

When the concentration of the standard (3.5 mg. of starch in the solution cell) was divided by the reading of the standard on the colorimeter scale, a factor was obtained. This factor multiplied by the scale reading of the solution being tested gave the amount of undigested starch remaining in the cell. This remaining starch was subtracted from 3.5 mg. (the original amount of starch in the cell) to give the quantity of starch digested during the test period. The average of 9 readings of the standard for each batch of starch was taken as the figure from which the factor was calculated. Each batch of starch was taken as a separate unit during the collection of data.

After the standards were determined the actual test followed. Forty ml. of starch solution containing 3.5 mg. of starch were measured from a burette into a clean dry cell 20 mm. in depth which was placed in the colorimeter. To this was added 0.1 ml. of unstimulated saliva fresh from the mouth of the individual donor. The mixture was given 10 rotatory stirs and the colorimeter adjusted to zero to eliminate the turbidity factor. Exactly 1 minute after the introduction of the saliva 0.025 ml. of 0.05 N iodine solution was added and again the cell contents were given 10 rotatory stirs. The reading was taken immediately (about 10–15 seconds) after adding the iodine. The addition of the iodine almost stopped digestion.

OUTLINE OF PROCEDURE

1. Switch colorimeter on 5 minutes before using.
2. Fill cell with 40 ml. of starch solution containing 3.5 mg. of starch.
3. Place cell in colorimeter so that light passes through 20 mm. of solution.
4. Add 0.1 ml. of fresh, unstimulated saliva.
5. Begin 1 minute interval of digestion.
6. Mix with 10 rotations of stirring rod during digestion.
7. Adjust colorimeter scale to zero during digestion.

8. At end of 1 minute add 0.025 ml. of iodine (0.05 N).
9. Mix with 10 rotations of stirring rod.
10. Read colorimeter and record reading.

TABLE I

Analysis of variance of amylolysis of the saliva of 6 individuals expressed in mg. of starch digested in 1 minute

	S.	C.	P.	M.	E.	R.	GROUP
No. readings	25	24	25	24	25	25	148
Mean amylolysis	3.30	2.72	2.34	1.90	2.75	3.26	2.72

SOURCE OF VARIATIONS	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES
Total	147	69.5212	
Between subjects	5	35.3751	7.075020
Between readings of each subject	142	34.1461	0.240465

F = 7.075020/0.240465 = 29.43 ..... significant
1% Significance = 3.14

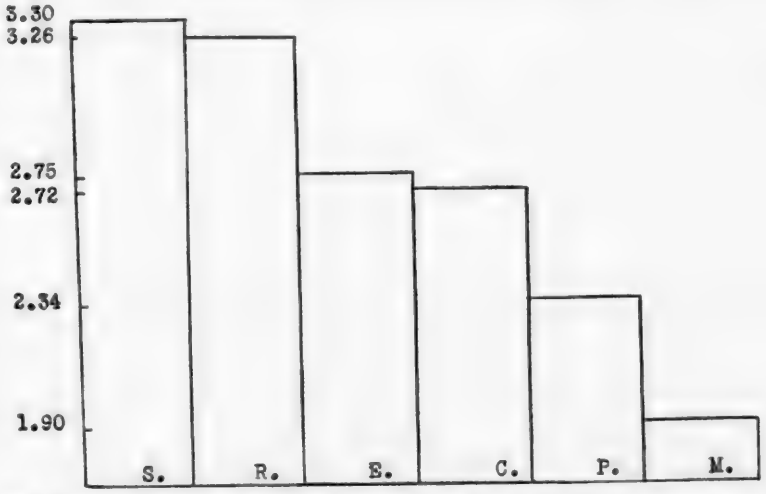

Fig. 1. Individual variation in the amylolysis of saliva expressed in mg. of starch digested in 1 minute.

DATA

Samples of unstimulated fresh saliva were collected from 6 persons between the ages of 34 and 48 years of age. The samples were taken at 9 and 11 a.m. and 1, 3 and 5 p.m. each day for 5 successive days. Although only 150 samples of saliva were used in the statistical analysis, about 450 samples were used in working out

the technic. The amounts of starch digested under the conditions of the experiment ranged from 0.38 to 3.34 mg. of the total of 3.5 mg. in the cell.

Referring to Table I, individuals S and R contributed saliva that digested almost all of the starch in the cell. E, C and P constituted a middle group and M's saliva digested an average of about half the starch in the cell. The analysis of variance between the salivas of various individuals (Table I) indicated that individual variations in the amylolytic activity of the various salivas were significant. *Fig. 1* illustrates graphically the individual variations of the group. The figures on the left side of the graph are mg. of starch digested.

Table II is an analysis of the bi-hourly variations in amounts of starch digested. These data are not significant.

TABLE II

Bi-hourly variations in the amylolysis of saliva

	9 A. M.	11 A. M.	1 P. M.	3 P. M.	5 P. M.	TOTAL
No. readings	29	30	30	30	29	148
Mean amylolysis	2.76	2.70	2.71	2.84	2.57	2.72

SOURCE OF VARIATIONS	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES
Total	147	69.5212	
Between bi-hourly periods	4	1.2064	0.301600
Between readings in each period	143	68.3148	0.477726

F = 0.477726/0.301600 = 1.58 ..... not significant
1% Significance = 13.57

DISCUSSION

The refinements in measuring amylolytic activity of saliva by the method presented herein are more valuable than the data obtained regarding the saliva of the indivuals studied. This method permitted the determination of ptyalin activity in terms of mg. of starch digested.

Other methods measure the end point of digestion in time, in the percentage of starch digested and in the various stages to which digestion has proceeded in a given time interval. None was based upon a technic that could check the standard or give a result in an absolute figure. It was laborious to spot-plate the sample repeatedly, as one method demands. End point determinations with the present colorimetric technic would be equally tedious. The use of ice baths and constant temperature baths and a number of tubes for each test made one method very cumbersome. Other methods utilized color which was determined visually and with doubtful accuracy. In contrast the technic presented herein avoided visual determinations, was fairly simple, used only 2 standardized solutions and gave a result in mg. of starch digested per unit time.

The sensitivity of the colorimeter disclosed considerable discrepancies in readings of the standard both within a single batch and even more within successive

preparations of starch solution. Five separate preparations of starch solution each tested 9 times during the period between 9 a.m. and 5 p.m. gave average standard readings on the colorimeter scale as follows: 345, 359, 366, 418, 421. The average for the entire 45 taken together was 380. Assuming that this approached the correct reading for 3.5 mg. of starch in 40 ml. of fluid with the light passing through a depth of 20 mm., the above reading of 345 would indicate that the cell contained 3.18 mg. while the cell reading 421 would contain 3.88 mg. of starch. That would be a difference of 0.7 mg. which is 20% of the total of 3.5 mg. of starch. The percentage of error is large, although the absolute error is quite small. However, it must be noted that previous methods of determining ptyalin activity did not attempt to ascertain variations in the standard starch solution.

There was no noticeable trends in the differences of the standard readings. This was true both within 1 solution as well as when comparing the readings of 2 different solutions of the same calculated strength. However, the variations within one batch of starch were not as great as the variations among successive batches. Within 1 batch the variations must have arisen from the inability of the operators to duplicate the technic within the limits of error demanded by the sensitivity of the colorimeter.

The variations between batches of starch solution were of greater magnitude and suggest errors in weighing, in putting the starch into solution and in quantitative dilution or they might indicate a lack of homogeneity in the starch reagent. Variations in the colorimeter were not considered. According to information furnished by the Klett Manufacturing Company the colorimeter is provided with 2 matched photoelectric cells of the blocking layer type in a fully compensated and carefully balanced electrical circuit. It was stated that this type of construction safeguarded the machine against fluctuations in light intensity due to power supply variations. Therefore the starch preparations rather than the instrument likely caused the error.

The data collected from the 6 individuals were in essential agreement with that offered by Myers and Adams (5) and Day (1). It indicated that there was considerable variation in the amylolysis of the saliva of various individuals. The data on variations in ptyalin activity throughout the day were not significant, but probably should be studied in more detail. The data of Gore (2), although taken for the entire 24 hour day was too limited in amount to be conclusive. A restudy of the problem in relation to other factors of saliva and to the incidence of caries using the technic described herein is contemplated. Added study of the technic seems necessary.

SUMMARY AND CONCLUSIONS

1. A method of estimating the amylolytic activity of saliva is described. 2. Soluble starch was weighed accurately and dissolved in water so that 40 ml. contained 3.5 mg. of starch. The indicator was 0.05 N iodine. 3. A Klett-Summerson photoelectric colorimeter with KS-54 filter was used to determine color intensity of the starch-iodine mixture. 4. To 40 ml. of starch solution in

a 20 mm. colorimeter cell was added 0.1 ml. of fresh saliva. The 2 solutions were mixed, the colorimeter was adjusted to zero and after 1 minute of digestion 0.025 ml. of iodine was added. After stirring, the amount of undigested starch was determined. This figure was subtracted from the original amount (3.5 mg.) to give the amount of starch actually digested. 5. This method measured amylolysis of saliva in terms of mgm. of starch digested under a standard set of conditions. 6. For 5 successive days fresh, unstimulated saliva of 6 individuals was tested for amylolytic activity at 9 and 11 a.m. and 1, 3 and 5 p.m. 7. Statistical analysis indicated that for the group the bi-hourly variations in amylolysis were not significant. 8. Variations among the 6 individuals in amylolytic activity of the saliva were statistically significant. 9. The technic needs additional refinement to take full advantage of the sensitivity of the photoelectric colorimeter.

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THE PATTERNS OF GROWTH AND DEVELOPMENT OF THE HUMAN TEETH AND JAWS[1]

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PART I. INTRODUCTION

The complexity of the problem under consideration is due to the concomitant and interrelated growth of the teeth and jaws. Previous studies have been largely concerned with the growth of teeth and jaws as an interrelated unit. This study recognizes that the teeth and the jaws have their respective independent patterns of growth and considers each separately. In this way the changing positional relationship of teeth and jaws during growth may be better understood. Previous studies have been largely concerned with the stage in development following the completed formation of the crown of the tooth. This study is largely concerned with the stage in development previous to the completed formation of the crown of the tooth.

With some important exceptions, there is, on the whole, a general agreement regarding the mechanism and the direction of growth of the jaw bones. Bone growth occurs by apposition and resorption. Very few observers today still adhere to the theory of interstitial phenomenon of bone growth. Experiments of the middle and late eighteenth century were, as a matter of fact, most convincing in nullifying the theory of the interstitial phenomenon of bone growth, although the notion was adhered to for many years afterwards and still is in some quarters. It is held chiefly with regard to some of the bones of the skull, if not directly, then by implication; it is involved with the difficulty of understanding the directions of growth of some of the bones of the skull, because of their complexity of relationship and arrangement.

It is necessary to consider the growth of the mandible in its two parts: that of the body and of the ramus. It is fairly well established that the body of the

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mandible increases in vertical dimension by apposition of bone, chiefly at the crest. Only about 10% of the vertical dimension of the body of the adult mandible is due to apposition of bone at its lower border (2, 3). *Fig. 1* is a composite diagram taken from Brash (2) and illustrates the increase in vertical dimension of the body of the mandible. Very little bone is added to the lower border of the mandible. Most of the bone is added gradually to the crest.

There is also general agreement that the increase in vertical dimension of the maxillae takes place by apposition of bone at the crest; but it is not at all clear what happens at the nasal border in the premaxillary region, or at the orbital borders of the bodies of the maxillae. Some observers believe that a certain amount of bone growth does take place in the superior borders, but that as bone growth occurs the entire maxilla is forced in a downward direction (7, 8). Other

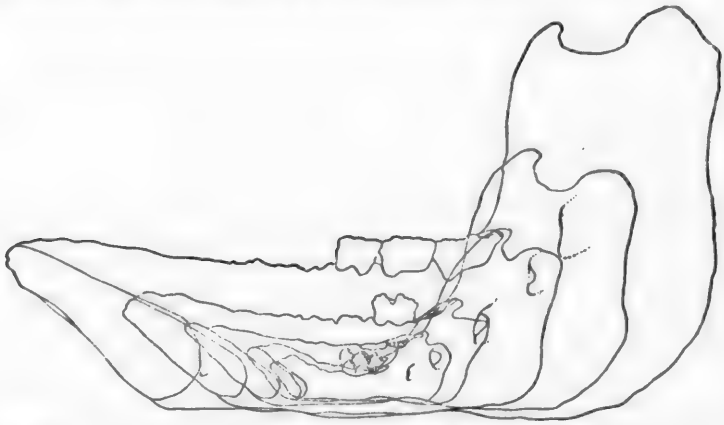


Fig. 1. Diagrammatic representation taken from Brash (2) showing direction of vertical growth of body of mandible of pig from birth to adult stage. The ages of the pigs were as follows: newborn, 4⅜ weeks, 11⅜ weeks, 32¼ weeks, 92⅜ weeks. The upward and forward movement is evident.

observers believe that the superior border of at least the premaxillary bone is a stable area (9, 23).

The Crown Germ

In discussing the manner and the direction of growth of the tooth germ it is necessary to clarify our views concerning the epithelial sheath, which outlines the forming tooth root, if we are to understand better the differences of opinion concerned with migratory movement.

Fig. 2 represents a tooth germ of a human mandibular first incisor at a familiar stage of development, often referred to as the "bell stage". There is as yet no evidence of hard tissue formation, although the outline in miniature of the future tooth is already descernible in the arrangement of the inner enamel epithelial cells, (A). These cells are actively concerned with the formation of enamel. The remaining epithelial cells—the super-structure of the enamel organ consisting of the stratum intermedium, (SI) which lies in several layers in close

proximity to the ameloblasts, the stellate reticulum (SR), and the outer enamel epithelial cells (OE)—play their respective parts in protecting and nourishing the enamel forming cells. At what may be regarded as the cervical end (CL), of the enamel organ, the inner and outer enamel epithelial cells join in a loop generally described as the epithelial sheath. In previous studies (11, 12), in collaboration with Applebaum, the author has demonstrated that at this stage of the development of the tooth there is as yet no evidence of an epithelial sheath. The cells of the enamel organ are entirely concerned with the formation of enamel. This stage in the development of the tooth should for accuracy be designated as the *crown germ*. We maintained in addition that the cervical

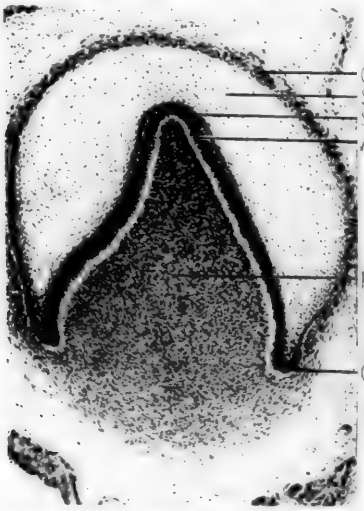


Fig. 2

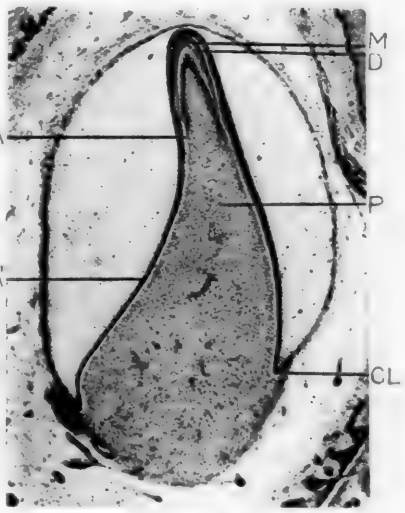


Fig. 3

Fig. 2. Crown germ of a deciduous mandibular first incisor just previous to hard tissue formation. Outline in miniature of future crown is discernible in arrangement of ameloblasts—A. OE—outer enamel epithelium; SR—stellate reticulum; SI—stratum intermedium; P—dental papilla; CL—cervical loop.

Fig. 3. Crown germ of deciduous mandibular first incisor showing beginning of hard tissue formation in incisal region. M—enamel matrix; D—dentin; A—differentiating ameloblasts; A'—undifferentiated ameloblats; CL—cervical loop; P—dental papilla.

ends of the enamel organ in the region of continuity between outer and inner enamel epithelium is the future cervical region of the crown, and should be designated as the *cervical loop*. The epithelial sheath, the outlining organ of the root, is not in evidence until the enamel matrix, with the exception of the small cervical region, is formed; and the epithelial sheath does not begin to function until most of the enamel is matured.

When Orban (23) observed that the epithelial sheath is a relatively fixed point, it is important to know that he used the term not alone for the true epithelial sheath, the formative organ of the root, but also for the cervical loop. His statement that the epithelial sheath is a relatively fixed point therefore applies

not only to the root stage of the development of the tooth, but also to the crown stage of its development. As his criterion he used the floor of the nose; therefore his investigation was concerned only with the maxillary incisor teeth. He noted that at any stage of the development of a tooth, whether in the crown or the root stage, the deepest point kept a relationship of proximity to the floor of the nose. He therefore concluded that the tooth germ, as it increased in vertical dimension, must be moving away from the fundus—in this case, the floor of the nose. This conclusion is necessarily based upon the belief that the floor of the nose is a stable area throughout the entire period of growth and development of the skull.

The question, specifically, is whether Orban's observation that "in the development of the tooth germs, there is no active inward growth taking place" (23) is correct. I will try to present evidence that this view is incorrect as far as the development of the crown is concerned, but, with some exceptions, is correct as far as the development of the root is concerned.

There is sufficient evidence that before the formation of hard tissues, the crown germ grows in length from the incisal or the occlusal region in the direction of the fundus (14, 21). With the beginning of enamel matrix formation, additional increase in length is obtained in the incisal or the occlusal region by the superimposition of the incremental layers of enamel matrix. The crown germ, therefore, increases its vertical dimension in both directions: in the incisal or the occlusal direction a prescribed amount, depending upon the thickness of enamel; and in the direction of the cervical region or the fundus, the remaining amount.

Fig. 3 represents a mandibular incisor at a somewhat later stage of development. In the incisal region, where enamel (M) is in the process of formation, the ameloblasts are functionally differentiated. No increase in vertical dimension can occur in this region except by superimposition of additional increments of enamel. But in the region below the terminal end of the formed enamel increment the ameloblasts (A) are still in a differentiating state; and evidences of mitotic figures are plentiful in all of the stages of the differentiating process of the cell, indicating a considerable increase by proliferation. Growth therefore must occur in the direction of the cervical loop (14, 21).

Materials of Study

Human embryologic specimens used ranged in age from ca. 4 to 7 months *in utero*, and were cut in sagittal, longitudinal, and frontal sections. Postnatal material ranged in age from birth to about 5 years of age. All sections are stained with hematoxylin-eosin. Human skulls studied were from about 7 months *in utero* to about $5\frac{1}{2}$ years of age. Included in this study are the human skulls from the collection of Dr. L. M. Waugh, for which due acknowledgment is made.

Findings

Fig. 4 is a low power view of human embryo jaws at about $4\frac{1}{2}$ months, cut in sagittal section. The maxillary (MC) and the mandibular (MI) deciduous

crown germs can be seen in their respective positions. Both crown germs are generally surrounded by their respective follicles (F). The follicle is an arrangement of loose connective tissue. The sagittal view of the entire mandible (M)

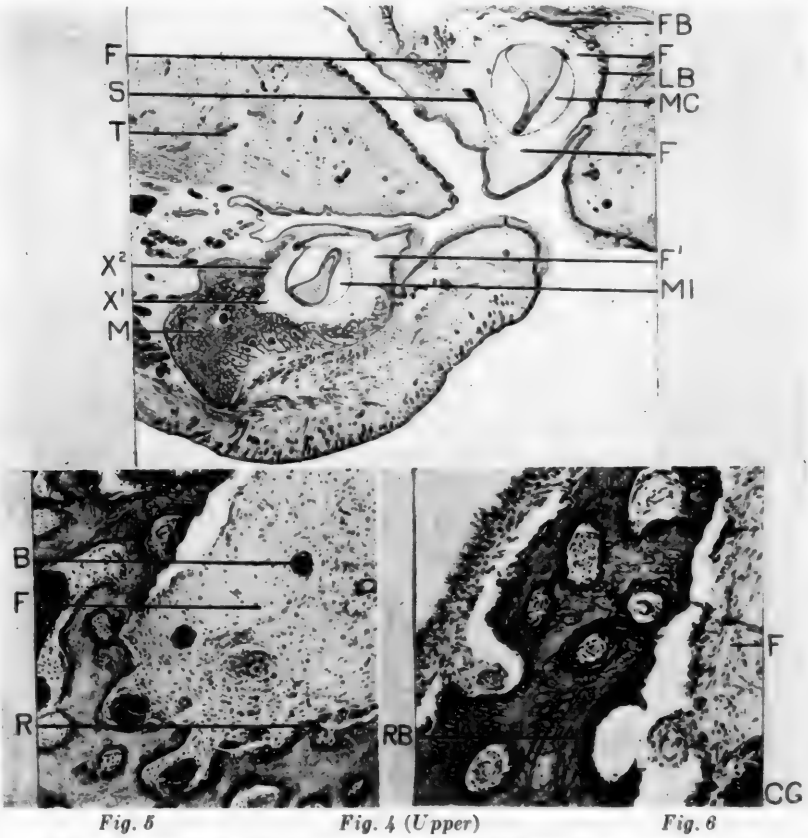


Fig. 5 *Fig. 4 (Upper)* *Fig. 6*

Fig. 4. Sagittal section of human embryo of about 4½ months showing maxillary (MC) and mandibular (MI) deciduous first incisor crown germs in their respective positions. Follicle (F) surrounds the maxillary crown germ and the follicle (F′) surrounds mandibular crown germ. FB—fundic bone of maxillary crown germ; LB—labial bone; S—successional lamina; T—tongue; X′—fundic bone of mandibular crown germ; X²—lingual bone of mandibular crown germ.

Fig. 5. Higher magnification of fundic bone (area X′ of *fig. 4*) showing resorption (*R*) on surface of bone adjacent to dental follicle (F). B—blood vessel.

Fig. 6. Higher magnification of lingual bone (area X² of *fig. 4*) showing resorption (RB) on inner surface adjacent to follicle (F). CG—crown germ.

is seen. No bone formation is present incisally above the maxillary or the mandibular crown germs. Resorption is evident on the inner aspects of the entire extent of bone adjacent to the follicles in both the maxilla and the mandible. *Fig. 5* is a higher magnification of the area (X′) of *fig. 4*. This includes

part of the lingual and part of the fundic bone of the mandibular crown germ. Resorption is evident on the side adjacent to the follicle (F). The follicle is

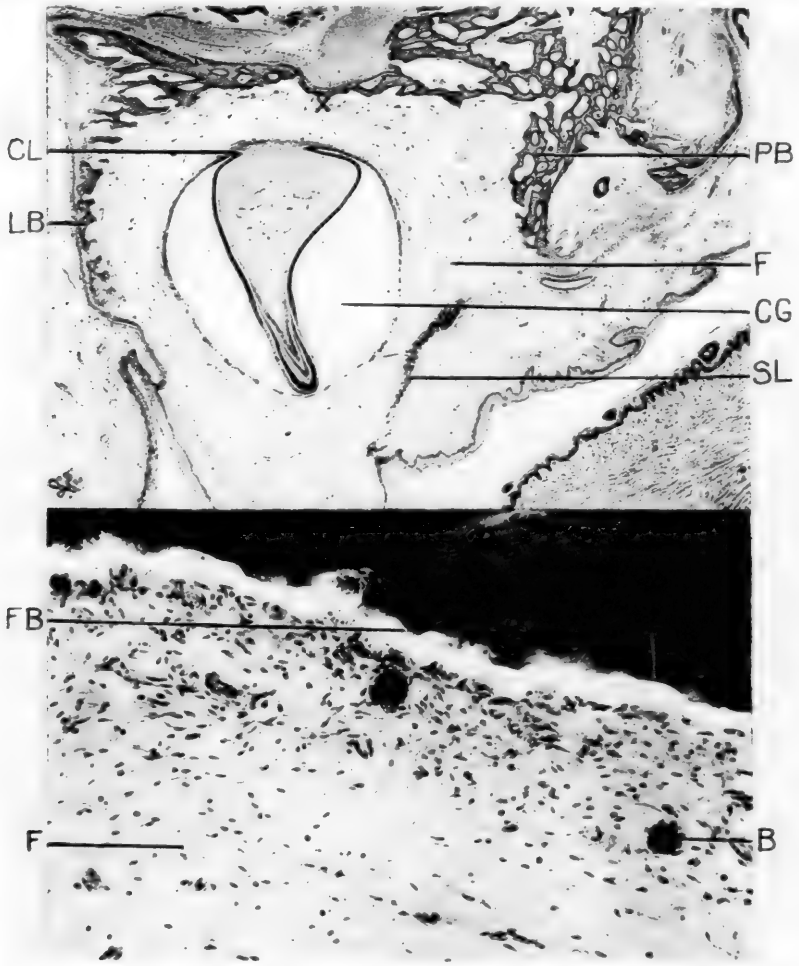


Fig. 7 (Upper) *Fig. 8 (Lower)*

Fig. 7. Sagittal section of human embryo of about 4½ months showing anterior region of maxilla with maxillary deciduous first incisor crown germ (CG) PB—palatal bone; LB—labial bone; F—follicle; CL - cervical loop; SL—successional lamina; X—fundic bone at floor of nose.

Fig. 8. Higher magnification of fundic bone (area X of *fig. 7*). Resorption at inner surface of fundic bone (FB) adjacent to follicle (F). B—blood vessel.

highly vascular. *Fig. 6* is a higher magnification of the area (X^2) in *fig. 4* of the lingual plate of bone of the mandibular incisor crown germ. There is evidence of resorption on the inner aspect adjacent to the follicle of the crown germ.

Fig. 7 is a low power magnification of the maxillary incisor crown germ (CG) surrounded by its follicle (F). *Fig. 8* is a higher magnification of the fundic bone (X) of *fig. 7* with evidence of resorption on the inner aspect adjacent to the

Fig. 10 *Fig. 9 (Upper)* *Fig. 11*

Fig. 9. Low power magnification of sagittal section of human embryo of about 5½ months with maxillary (CG) and mandibular first incisor crown germs. LB—labial bone; F—follicle; X—region of labial crest; X′—labial bone; X²—fundic bone at floor of the nose; T—tongue; M—mandible.

Fig. 10. Higher magnification of labial crest of bone (area X of *fig. 9*) showing active apposition of bone chiefly at the labial side of the crest (C). Active apposition extends along the entire labial region (L) of the labial bone.

Fig. 11. Higher magnification of area X′ of *fig. 9*. This is labial bone directly adjacent to the area shown in *fig. 10*. Active bone growth is occurring all along line on labial (L) or outer region of bone. Inner surface adjacent to the follicle (F) is old bone (O) largely at rest, with slight evidence of resorption (R).

follicle and new bone formation on the opposite side toward the nose. The follicle is highly vascular.

Fig. 12 (Upper) *Fig. 13 (Lower)*

Fig. 12. Higher magnification (area X^2 of *fig. 9*) of bone at fundus. There is resorption (R) of inner aspect of fundic bone and apposition of new bone (NB) in direction of floor of nose. Follicle (F) is highly vascular with some evidence of extravasation. CG—crown germ.

Fig. 13. Higher magnification (area X^3 of *fig. 9*) of lingual bone near crest and including palate side. Active bone growth may be seen at the crest (C) and on palate side (P) and bone resorption on inner side of bone trabeculae adjacent to follicle. CG—crown germ; F—follicle; R—resorption; NB—new bone formation.

Fig. 9 is a low power magnification of a sagittal section near the midline of a human embryo at about $5\frac{1}{2}$ months with maxillary (CG) and mandibular first incisor crown germs in their respective positions. The crown germs are sur-

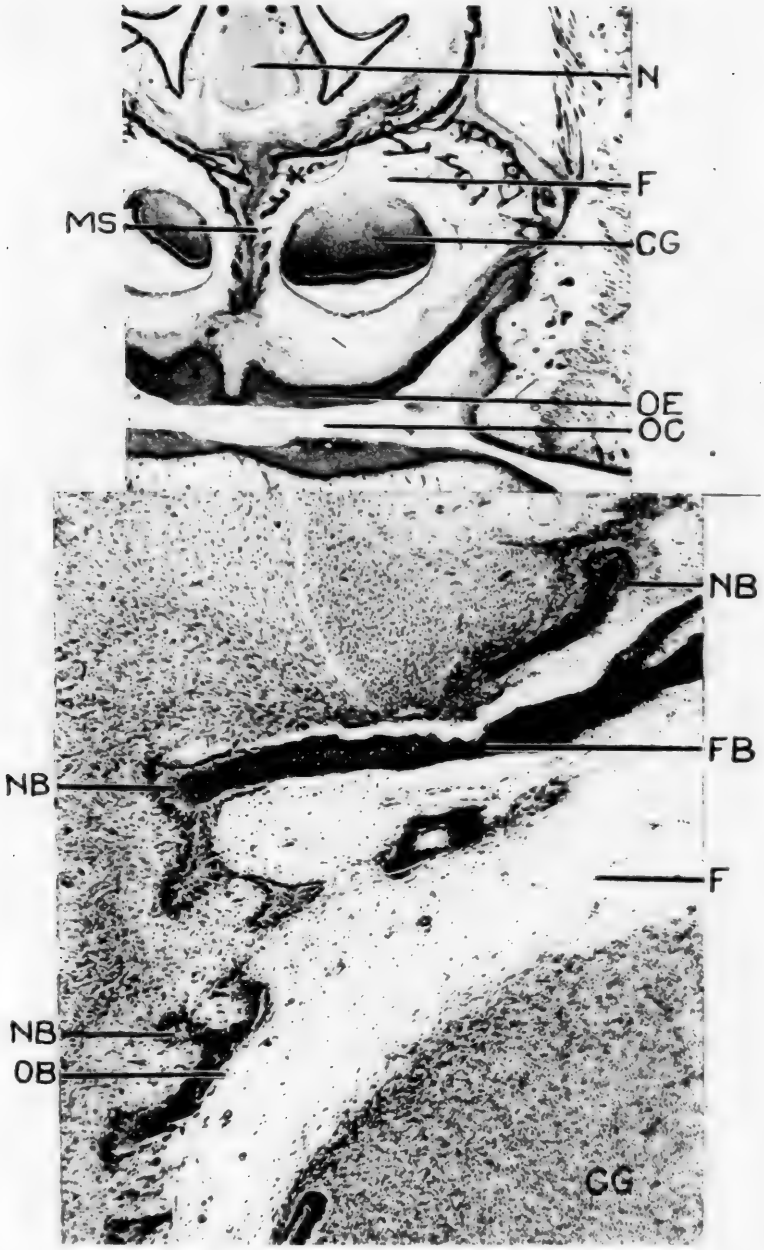


Fig. 14 (*Upper*) *Fig. 15* (*Lower*)

Fig. 14. Low power view of frontal section of human embryo at about 4½ months, cut through area of maxillary deciduous crown germ (CG) and nose. Crown germ is surrounded by loose connective tissue (F). Bone is present at fundic area of floor of nose as well as on distal and mesial aspects. There is an absence of bone incisally above crown germ. Mesially (MS) is the maxillary suture. X—cartilage; OE—gingival epithelium; OC—oral cavity.

Fig. 15. Higher magnification of fundic bone (area X in *fig. 14*), which extends mesially to maxillary suture and includes part of mesial bone. Fundic bone (FB) adjacent to follicle appears to be inactive. Apposition of new bone may be seen on side toward nose (NB). At mesial terminal end of fundic bone, new bone formation is evident in direction of maxillary suture. New bone formation is also evident on mesial side of mesial bone in direction of maxillary suture, and old bone adjacent to follicle (F). CG—crown germ.

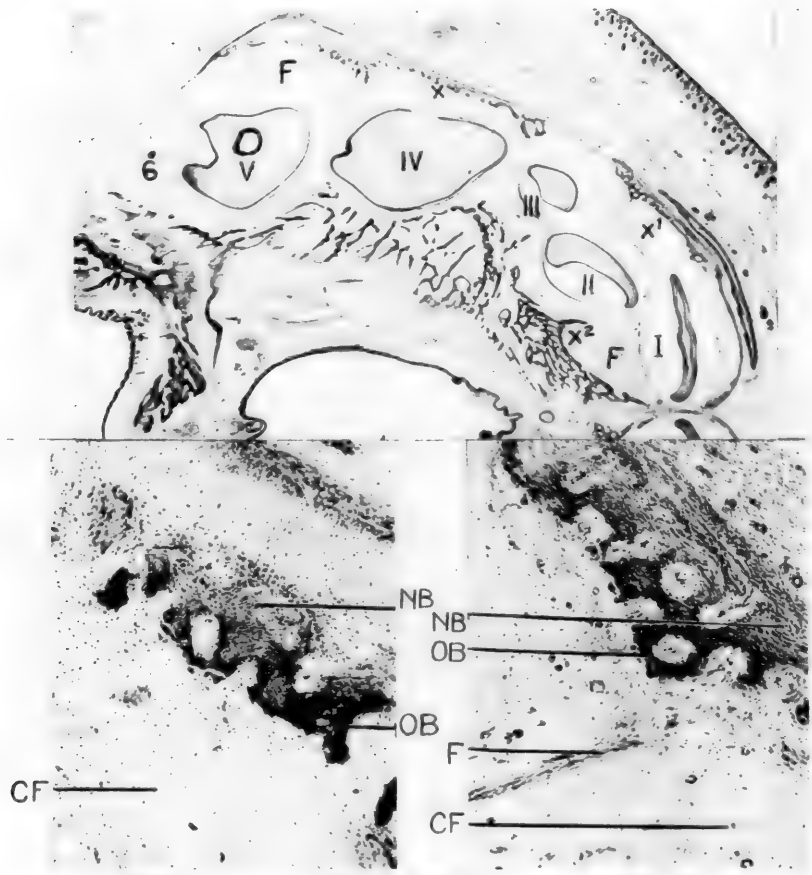


Fig. 17 *Fig. 16 (Upper)* *Fig. 18*

Fig. 16. Low power view of horizontal section of maxilla of embryo at about 6 months. Horizontal views of all of 5 deciduous maxillary and first permanent molar crown germs are seen in their respective states of development. Second incisor crown germ (II) appears in lingual relation and crowded between the first incisor (I) and the canine crown (III) germs. Follicle (F) surrounds individual crown germs. Thin layer of bone borders buccal and labial sides of follicles, with exception of canine area. Varied thicknesses of bone border lingual or palatal side of follicle. IV—first deciduous molar crown germ; V—second deciduous molar crown germ; 6—first permanent molar crown germ; X—buccal bone in the the region of first deciduous molar crown germ; X′—labial bone adjacent to second incisor and canine crown germs; X^2 lingual or palatal bone adjacent to first and second incisor crown germs.

Fig. 17. Higher magnification of bone on buccal side (area X of *fig. 16*) of deciduous first molar crown germ. Apposition of bone is evident on outer or buccal side (NB) of small trabeculae, and resorption (OB) on inner side adjacent to follicle. Young connective tissue fibers (CF) are seen in profusion at outer rim of follicle (F).

Fig. 18. Higher magnification of labial bone (area X′ of *fig. 16*) adjacent to follicles of canine and second incisor crown germs. Bone apposition (NB) is evident at outer or labial aspect of bone, and resorption (OB) of inner aspect is seen adjacent to follicle. CF—connective tissue fibers bordering the follicles of second incisor and canine crown germs.

rounded by their respective follicles (F). There is as yet no bone formation incisally above the crown germs. The bone surrounds the follicle on only 3 aspects—the labial side, the lingual side, and at the fundus. *Fig. 10* is a magnification of the region of the crest (area X of *fig. 9*) of the labial bone of the maxillary crown germ. New bone growth is very active at the outer or labial (C) side of the crest. New bone formation extends along the outer or the labial (L) side of the labial bone. *Fig. 11* is a higher magnification of area X′ of *fig. 9*, the labial bone directly adjacent to the area shown in *fig. 10*. Active bone growth is occurring all along the line on the labial or outer region of the bone. The inner surface adjacent to the follicle (F) is old bone largely at rest, with slight evidence of resorption. *Fig. 12* is a higher magnification (area X^2 of *fig. 9*) of the bone at the fundus. There is resorption (R) of the inner aspect of the fundic bone and apposition of new bone (NB) in the direction of the floor

Fig. 19. Higher magnification of the area (X^2 of *fig. 16*) on palatal side of deciduous first and second incisor crown germs. Resorption (OB) is evident on inner aspect of bone adjacent to follicle (F), and apposition (NB) on palatal side of bone.

of the nose. The follicle is highly valcular. *Fig. 13* is a higher magnification (area X^3 of *fig. 9*) of the lingual bone near the crest and including the palate side. Active bone growth may be seen at the crest (C) and on the palate side (P), and bone resorption on the inner side of the bone trabeculae adjacent to the follicle.

Fig. 14 is a low power view of a frontal section of a human embryo at about 4½ months, cut through the area of the maxillary deciduous crown germ (CG) and the nose. The crown germ is surrounded by loose connective tissue (F). Bone is present at the fundic area of the floor of the nose as well as on the distal and mesial aspects. There is an absence of bone incisally above the crown germ. Mesially (MS) is the maxillary suture. *Fig. 15* is a higher magnification of the fundic bone (area X in *fig. 14*), which extends mesially to the maxillary suture and includes part of the mesial bone. The fundic bone (FB) adjacent to the follicle shows evidence of resorption. Apposition of bone may be seen on the

side toward the nose (NB). At the mesial terminal end of the fundic bone, new bone formation is evident in the direction of the maxillary suture. New bone formation is also evident on the mesial side of the mesial bone in the direction of the maxillary suture, and old bone adjacent to the follicle.

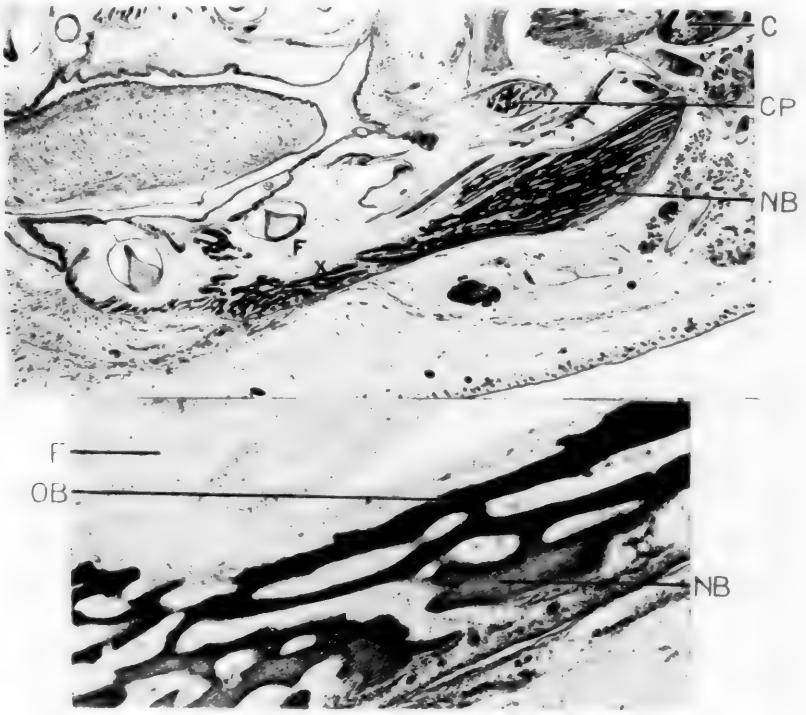


Fig. 20 (Upper) *Fig. 21 (Lower)*

Fig. 20. Low power view of sagittal section of human embryo at about 6 months. Four of crown germs, second incisor, first and second deciduous molars, and first permanent molar are seen in this section in their respective stages of development in mandible. There is no evidence of bone anywhere along crest of these crown germs. Bone outlines lower border of the mandible and part of the ramus. Head of condyle (C) and coronoid process (CP) are also seen. Entire interior of body of mandible with part of anterior border of ramus consists of loose connective tissue, with generous spacing between developing tooth germs, as noted particularly between first and second deciduous crown germs. Apposition of bone (NB) is evident in posterior border of ramus. X—fundic bone of deciduous mandibular first molar crown germ.

Fig. 21. Higher magnification of the fundic bone (area X of *fig. 20*) of deciduous mandibular first molar, including lower border of mandible. Resorption of bone (OB) is seen on inner aspect adjacent to follicle (F) of crown germ, and considerable bone formation (NB) at lower border of mandible.

Fig. 16 is a low power view of a horizontal section of the maxilla of an embryo at about 6 months. Horizontal views of all of the 5 deciduous maxillary and first permanent molar crown germs are seen in their respective stages of development. The second incisor crown germ (II) appears in lingual relation and

crowded between the first incisor (I) and the canine[2] crown (III) germs. The follicles (F) surround the individual crown germs. A thin layer of bone borders the buccal and labial sides of the follicles, with the exception of the canine area. Varied thicknesses of bone border the lingual or palatal side of the follicle.

Fig. 17 is a higher magnification of the bone on the buccal side (area X of *fig. 16*) of the deciduous first molar crown germ. Apposition of bone is evident on the outer or buccal side (NB) of the small trabeculae, and resorption (OB) on the inner side adjacent to the follicle. Young connective tissue fibers (CF) are seen in profusion at the outer rim of the follicle (F). *Fig. 18* is a higher magnification of the labial bone (area X′ of *fig. 16*) adjacent to the follicles of the canine and second incisor crown germs. Bone apposition (NB) is evident at the outer or labial aspect of the bone, and resorption (OB) of the inner aspect is seen adjacent to the follicle. *Fig. 19* is a higher magnification of the area (X² of *fig. 16*) on the palatal side of the deciduous first and second incisor crown germs. Resorption (OB) is evident on the inner aspect of the bone adjacent to the follicle (F), and apposition (NB) on the palatal side of the bone.

Fig. 20 is a low power view of the sagittal section of a human embryo at about 6 months. Four of the crown germs, the first incisor, the first and second deciduous molars, and the first permanent molar, are seen in this section in their respective stages of development in the mandible. There is no evidence of bone anywhere along the crest of these crown germs. Bone outlines the lower border of the mandible and part of the ramus. The head of the condyle (C) and the coronoid process (CP) are also seen. The entire interior of the body of the mandible, with part of the anterior border of the ramus, consists of loose connective tissue, with generous spacing between the developing tooth germs, as may be noted particularly between the first and second deciduous molar crown germs. Even in this low power magnification apposition of bone (NB) is evident in the posterior border of the ramus. *Fig. 21* is a higher magnification of the fundic bone (area X of *fig. 20*) of the deciduous mandibular first molar, including the lower border of the mandible. Resorption of bone (OB) is seen on the inner aspect adjacent to the follicle (F) of the crown germ, and considerable bone formation (NB) at the lower border of the mandible.

Discussion

Evidence has been presented to demonstrate the manner and direction of growth of the crown germs. The connective tissue follicle (25) has not been given its proper significance in the study of the growth process of the crown germ. The evidence indicates that the connective tissue follicle is in an active period of growth, particularly during the early period of the development of the crown germ. This is indicated partly by the strands of young connective tissue fibers at the peripheral borders of the growing follicle, and partly by the resorption of bone on the surfaces directly adjacent to the follicle. Growth of the con-

[2] The author prefers the term "cuspid" when referring to human material but the term "canine" is used throughout this paper in accordance with the custom of this Journal and the recommendation of the Nomenclature Committee of the American Dental Association (Ed.)

nective tissue produces the resorption of bone in all directions, including the direction of the fundus, and is always in advance of the growth of the crown germ. The growth of the follicle occurs apparently with some intervals of rest, and continues until sufficient room has been established for the completed formation of the future crown of the tooth. The epithelium of the crown germ invades the connective tissue follicle in all directions, including the direction of the fundus. There is no evidence that the crown germ migrates from its original site during its development in any direction, buccally, lingually, or in the direction of the crest of the jaw. The crown germ develops, instead, in a fixed site, increasing its size eccentrically in all directions according to its pattern, the path being cleared for it by the advance growth of the connective tissue follicle.

Evidence was also presented that bone growth occurs by apposition at the outer surfaces buccally, lingually, mesially, and at the labial or outer aspects of the crest. It has been possible to demonstrate the occurrence of these phenomena in three directions, sagittal, horizontal, and frontal.

Sutural Growth

To understand the manner and direction of growth of the jaw bone, it is important to visualize growth of the bone in three dimensions. The bone grows simultaneously in all directions. By so visualizing one can more easily understand what happens at a suture. As growth of bone occurs labially, the perimeter of the anterior curve is steadily increased, widening the suture area into which bone grows from the mesial terminal ends of the bone. This was demonstrated in *fig. 15*, a frontal section which included the maxillary suture. Therefore, there is no spreading of the maxillae laterally by addition of bone at the suture. The lateral widening of the maxillae also occurs by the processes of apposition and resorption. The concept that sutural bone growth increases dimensions by spreading the bones adjacent to a suture, is untenable.

No Cause and Effect Relation

There is no evidence of a cause and effect relation between bone growth and crown germ development. Growth of the crown germ does not influence bone growth at the crest, which occurs simultaneously outwardly in a labial or a buccal direction and vertically in the direction of the oral cavity.

PART II. ANATOMIC CORRELATION

The histologic findings may be correlated with anatomic evidence.

Fig. 22 shows a maxilla (A) and a mandible (B) of a human at birth; the crown germs are removed. The body of the mandible and the dental process of the maxillae consist of crypts of the deciduous crown germs and their follicles. The crypts (6) of the first permanent molar crown germs are also in evidence. The bone consists of exceedingly thin plates which surround the individual crown germs. There is no bone as yet at the crest covering the crown germs incisally or occlusally. The greatest thickness of bone is at the lower border of the body of the mandible. This measures 2½ mm. from the fundus of the second deciduous molar to the lower border of the body of the mandible; 6 mm. from

the fundus of the deciduous first incisor to the lower border of the mandible; and $7\frac{1}{2}$ mm. from the fundus of the first permanent molar crypt to the lower border of the mandible. Measurement of all diameters of the crypts of the first and second deciduous molar crown germs reveals that the maximum space

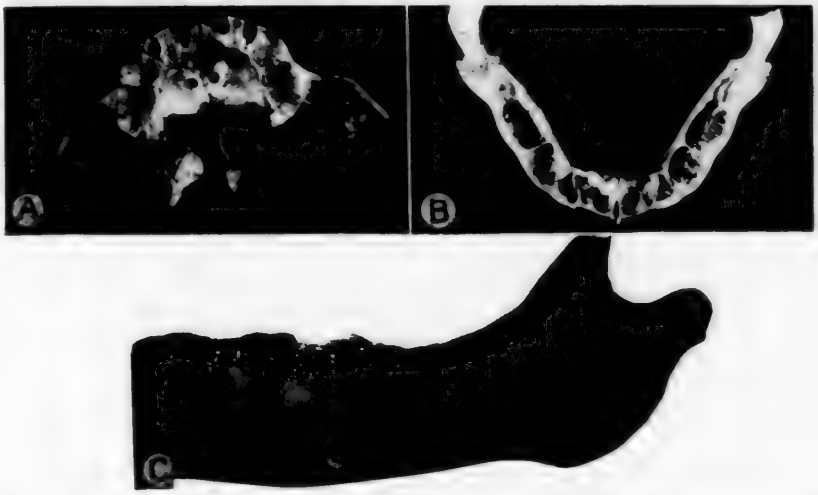


Fig. 22. A—occlusal view of a human maxilla at birth. B—occlusal view of a human mandible at birth. C—lateral view of a human mandible at birth.

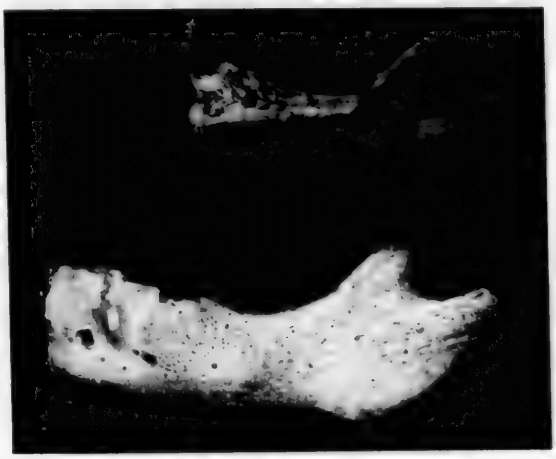


Fig. 23. Lateral views of a human mandible about 7 months *in utero* and of a human mandible at birth.

for the first and second deciduous molar crowns is already attained, although the crowns of these teeth are far from completed at birth. At this stage, hard tissue formation has just started at the dentino-enamel junction of the future longest cusp of the first permanent molar crown germ. Although the crypt

is much smaller than the completed crown of the tooth, it is still considerably larger than the crown germ. The crypt extends from the crest of the bone in the direction of the lower border of the mandible. *Fig. 22* C is a lateral view of the mandible, showing the porosity and the thinness of the buccal plates of bone, and the outlines of the crypts of the deciduous crown germs and of the first permanent molar (6) which at this stage of development is still considerably removed from the lower border of the mandible.

Fig. 23 is a lateral view of human mandibles, one at about seven months *in utero*, and the other at the birth stage; it shows the comparative size of their development. The rami appear in fairly wide obtuse angle relation to the body of their respective mandibles.

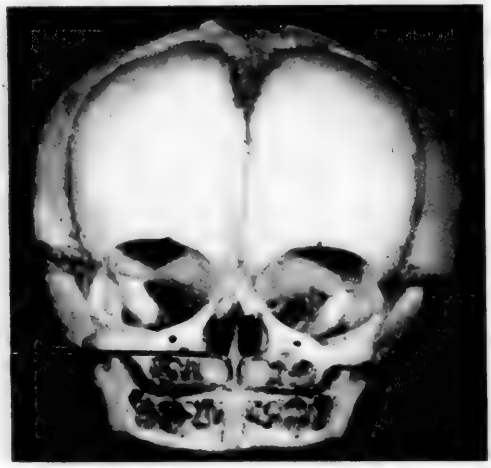


Fig. 24. Frontal view of a human skull at birth. F—fundic bone of deciduous maxillary first incisor crown.

Fig. 24 is an anterior view of another human skull at birth. The labial plates of bone have been removed; the deciduous maxillary incisor teeth occupy the entire vertical dimension of the premaxillary bone. At birth, the enamel matrices of the maxillary deciduous first incisor teeth are completed or nearly completed; the crown may therefore be said to have attained its maximum dimensions. The contour of the fundic bone (F) of the deciduous first incisor crowns follows the contour of the cervical region of the crown of the tooth very closely; the convexity extends into the nasal cavity. The second incisor crown germ is in a position slightly lingually to the first incisor. These findings are similar to the histologic evidence presented in the horizontal section, *fig. 17*.

Fig. 25 is a lateral view of a human skull at about 9 months of age, with the outer plates of bone removed to disclose the developing deciduous teeth. Bone is present all along the crest, covering the incisal and occlusal surfaces of the developing crowns. The crowns of the deciduous first molar teeth (IV) are completed or nearly completed. Their occlusal surfaces lie close to the crest

bone, and bone is in close proximation to the mesial and distal surfaces of the crown. The follicle has disappeared, except in the cervical region near the fundus. The mandibular second deciduous molar crown (V) is far from completion.

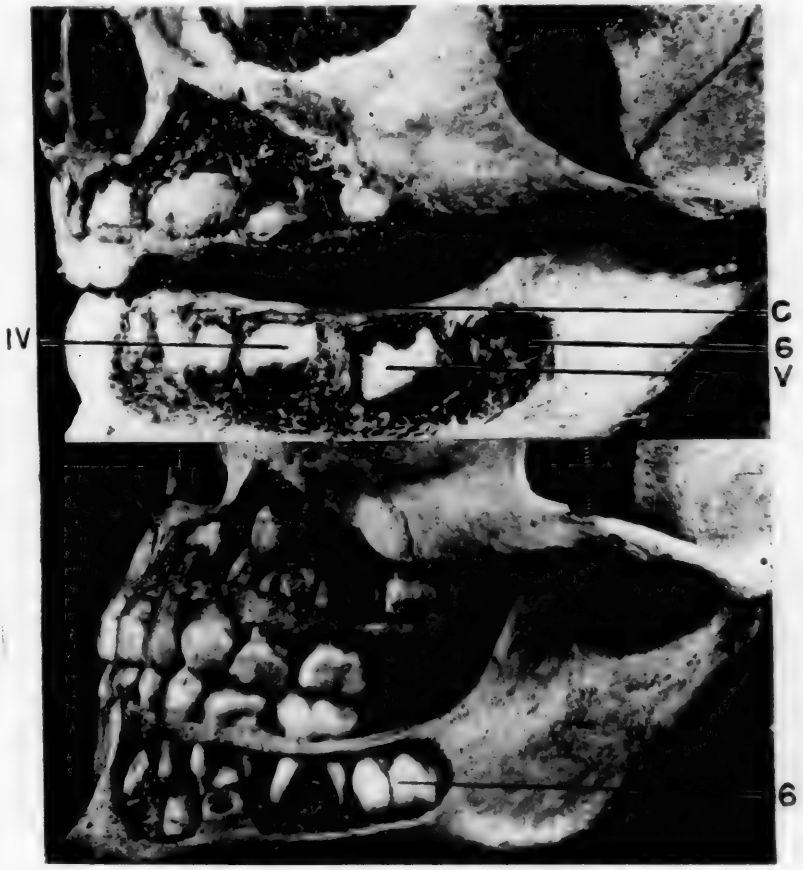


Fig. 25 (Upper) *Fig. 26 (Lower)*

Fig. 25. Lateral view of a human skull at 9 months of age with outer or buccal plates of bone removed disclosing crowns of deciduous and first permanent molar crown germs. IV—mandibular first deciduous molar crown; V—mandibular second deciduous molar crown; 6—crypt of mandibular first permanent molar crown germ; C—crest of bone.

Fig. 26. Lateral view of a human skull 2 years and 4 months of age with the outer or buccal plates of bone removed, disclosing developing permanent crown germs. 6—mandibular first permanent molar crown.

The enamel is still in a matrix state; the occlusal morphology indicates incompleteness of formation. The developing crown is freely surrounded by a space, including the region between the crest of bone and the incomplete occlusal surface of the crown. This space indicates the presence of the follicle.

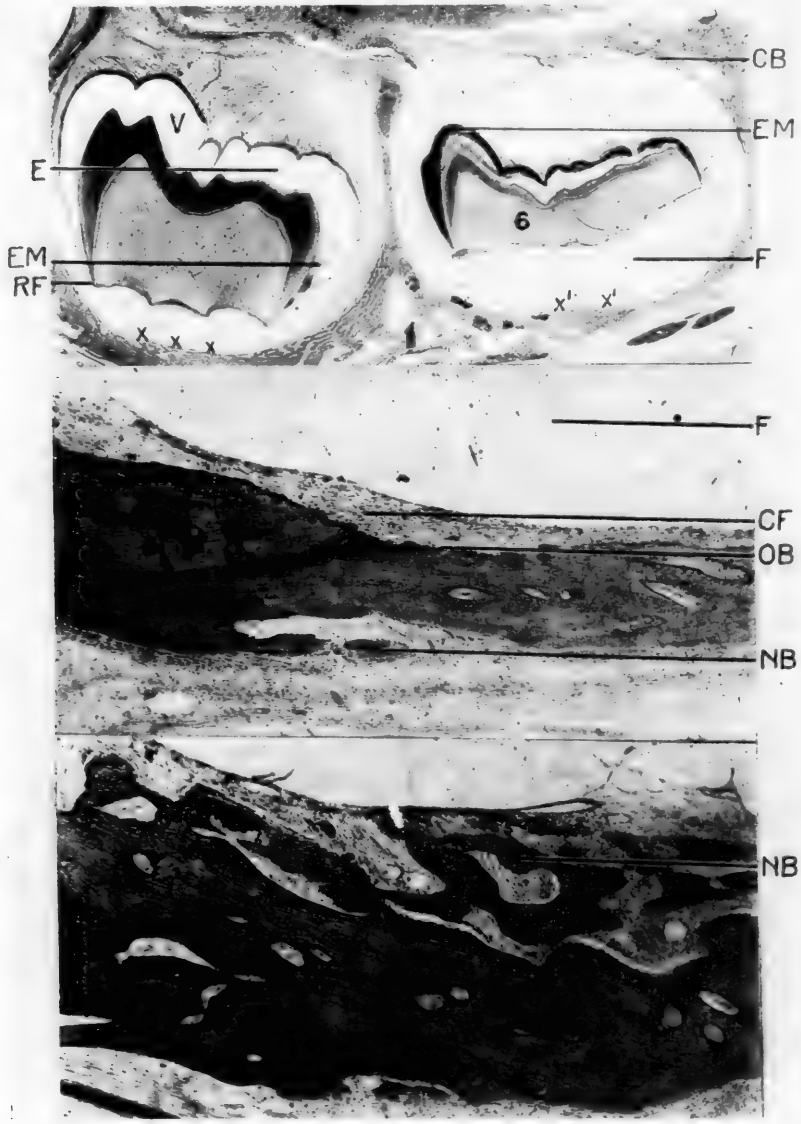


Fig. 27 (*Upper*) *Fig. 28* (*Center*) *Fig. 29* (*Lower*)

Fig. 27. Sagittal section of human mandible about 9 months of age with the deciduous second molar tooth (V) and first permanent molar crown in their respective stages of development. Crown of the second deciduous molar is completed. Enamel (E) is almost entirely calcified and part of root (RF) is already formed. Remains of follicle (F) are seen in region of fundus. Crown of first permanent molar is still in process of formation. Enamel (EM) is in matrix state and enamel matrix is not yet completely formed. Bone surrounds follicle (F) on all sides, including area of crest (CB) on distal region above first permanent molar. RF—root formation; 6—first permanent molar crown germ; CB—crest of bone. This specimen courtesy Dr. Isaac Schour.

Fig. 28. Higher magnification of fundic bone (area X′ of *fig. 27*) of developing crown of first permanent molar. Resorption (OB) of bone is evident on inner aspect of fundic bone adjacent to follicle (F) of developing first permanent crown, and apposition (NB) is seen on outer rim. CF—connective tissue fibers.

Fig. 29. Higher magnification of fundic bone (area X of *fig. 27*) of completed crown of second deciduous molar, with beginning of root formation. Apposition of bone (NB) is in evidence on inner aspect of fundic bone adjacent to root of tooth.

Fig. 26 is a lateral view of a human skull at 2 years and 4 months of age. The crown of the first permanent molar (6) is fairly well completed, although maturation of the enamel is still in progress. The cervical region is very close to the lower border of the mandible as compared to the birth stage previously shown, where the fundus of the first permanent molar was 7½ mm. removed from the lower border of the mandible. The crown is completely surrounded by bone, which is in close proximity to the peripheral surfaces of the crown. The thickness of bone at the crest is greater than at the 9 months old stage.

Fig. 27 is a sagittal section of a human mandible at about 9 months of age with the deciduous second molar tooth (V) and first permanent molar crown in their respective stages of development. The crown of the second deciduous molar is completed. The enamel (E) is almost entirely calcified and part of the root (RF) is already formed. The remains of the follicle are seen in the region of the fundus. The crown of the first permanent molar is still in the process of formation. The enamel (EM) is in the matrix state, and enamel matrix is not yet completely formed. There is still evidence of the follicle (F) surrounding the developing crown. Bone surrounds the follicle (F) on all sides, including the area of the crest (CB) on the distal region above the first permanent molar. *Fig. 28* is a higher magnification of the fundic bone (area X′ of *fig. 27*) of the developing crown of the first permanent molar. Resorption (OB) of bone is evident on the inner aspect of the fundic bone adjacent to the follicle (F) of the developing first permanent crown, and apposition (NB) is seen on the outer rim. *Fig. 29* is a higher magnification of the fundic bone (area X of *fig. 27*) of the completed crown of the second deciduous molar, with evidence of the beginning of root formation. Apposition (NB) of bone is in evidence on the inner aspect of the fundic bone adjacent to the root of the tooth.

Discussion

The anatomic evidence just presented may be correlated with the histologic evidence. Even in the birth stage there is no evidence of bone at the crest in any of the deciduous crown germs or in that of the first permanent molar. In a wet specimen with the soft tissues in place, part of the connective tissue follicle would be found orally above the existing crest of the bone. This is also seen in histologic sections (*fig. 4*). Crypts of the deciduous molar teeth at birth already answer to the dimensions, in all directions, of the completed crowns of the deciduous molar teeth, although the crown germs are far from a state of completion. The dimensions of these crypts have been attained by the growth of the follicle. The crypts extend from the crest of the bone to within 2½ mm. of the lower border of the mandible. Growth of the follicle is therefore in the direction of the lower border of the mandible deeper within the jaw bone. The buccal and lingual plates of bone simultaneously are increasing their vertical dimensions by additional formation of bone along the crest.

This manner and direction of growth is also apparent with the first permanent molar crown germ. At the birth stage the crypt is still quite small, indicating that the follicle has by no means attained its maximum size. Its location, how-

ever, is from the crest of the bone in the direction of the lower border of the mandible, but its fundus is about 7½ mm. from the lower border. As continued growth of the follicle occurs, bone surrounding the first permanent molar crown germ will be continuously resorbed into the region of the fundus, so that the fundus of the crown germ will be found closer to the lower border of the mandible.

This is seen happening in the 9 months old skull, where a number of important changes in the growth process are evident. The formation of the crowns of the deciduous first molar teeth is virtually completed, and the follicles have disappeared, with the exception of the region of the fundus, bone now lying in close proximity to the surfaces of the crown. Bone has continued to grow vertically in the direction of the crest; the occlusal surfaces of the crowns and crown germs are covered by bone. This evidence leads to the conclusion that bone growth along the crest of the body of the mandible and of the dental process of the maxillae, *follows an individual pattern which is in no way influenced by or dependent upon the growth and development of the teeth.* The mandibular second deciduous molar is in the state of formation; the follicle is considerably reduced and has undoubtedly stopped growing, because the maximum space for the completed formation of the crown is already established. The crypt of the first permanent molar is larger than at the birth stage, and the fundus is nearer the lower border of the mandible. The evidence of bone at the crest covering the occlusal surface region is just another indication of the continued growth of bone in the vertical direction.

In the 2 years and 4 months stage, the formation of the enamel matrix of the first permanent molar is completed. The cervical region of the crown is now quite close to the lower border of the mandible; there certainly can be no question what the direction of growth of the tooth germ was. The bone surrounds the crown of the tooth closely in all direction, including the occlusal surface. This phenomenon is substantiated by the histologic section of a deciduous second mandibular molar and a first permanent mandibular molar adjacent to it. The crown of the second deciduous molar is not only completed in formation, but is almost entirely calcified; and the root is in the process of formation. The follicle has disappeared, and bone lies in close proximity to the surfaces of the crown. Now that the follicle has stopped growing there is evidence of bone apposition on the inner surfaces of the fundus adjacent to the root of the tooth. The processes of bone apposition and resorption are reversed, and the crown of the tooth is now for the first time in a migratory stage.

These conditions are in striking contrast to those of the adjacent developing crown of the first permanent molar tooth. The follicle is still present and has perhaps reached its maximum size, although there is still some evidence of growth at the periphery and at the fundus, where resorption is present on the inner surface of the fundic bone adjacent to the follicle. The crown of this tooth is still developing in its fixed site, growing eccentrically in all directions, now largely by a superimposition of increments of enamel.

These phenomena of the growth of the crown germ deeper in the jaw are likewise evident for the maxillary incisor teeth, where the anatomic evidence

shows a thin plate of bone following the contour of the cervical region of the crown, the convexity of which extends into the nose. The histologic evidence of earlier stages supports the observation.

An additional piece of anatomic evidence is presented in *fig. 30*. This is a frontal view of the skull of a dwarf of about 5½ years of age, previously published by Broadbent (5). The maxillary permanent first incisor crowns are completed in formation; their cervical regions extend through the floor of the nose at (1) and into the nasal cavity. This phenomenon has been explained as failure of migratory vertical movement of the crown during its growth. It indicates, instead, a failure of bone growth at the floor of the nose, the crown continuing its growth deeper in the jaw and thereby reaching into the nasal cavity.

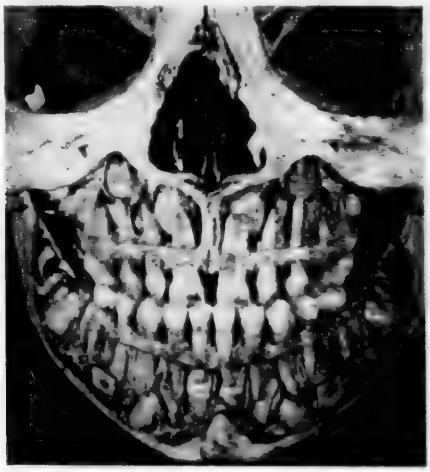


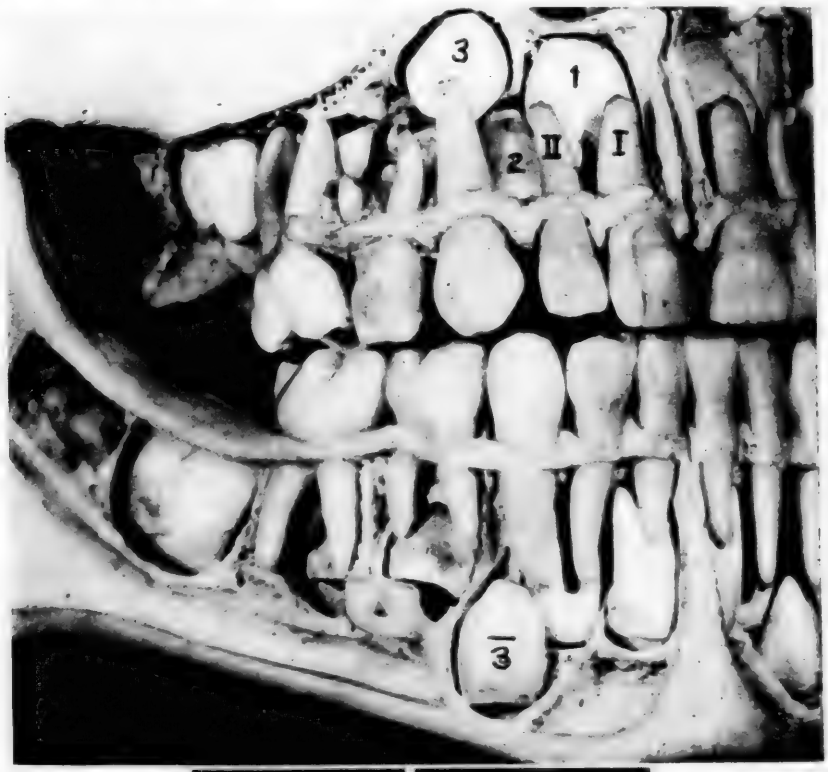
Fig. 30. Frontal view of skull of dwarf of about 5½ years of age. I—cervical region of maxillary permanent first incisor crown extending into nasal cavity. (Courtesy of Dr. Holley Broadbent.)

PART III. CHANGING POSITIONAL RELATIONSHIPS BETWEEN DECIDUOUS AND PERMANENT ANTERIOR TEETH

Upon the foregoing anatomic evidence, the changing positional relationships between the deciduous and the permanent anterior teeth in relation to the jaw bone can be readily deduced.

Fig. 31 is a lateral view of a human skull at about 5½ years of age. The outer plates of bone have been removed from the maxilla and the mandible, with the exception of a narrow rim at the crest. The roots of the deciduous as well as the crowns of the permanent anterior teeth are disclosed. The mandibular permanent canine crown (3) is located deep in the mandible, with its cervical region close to the lower border of the mandible. The permanent canine crown is completely formed and calcification is well advanced. The bone lies in close proximity to the surfaces of the crown in all directions. The crest of the bone is considerably above the incisal margin of the mandibular permanent cuspid crown.

One may readily visualize the sequence of events during growth that resulted in the anatomic relation between the crown of the permanent canine tooth, the crest of the bone, and the position of the deciduous canine tooth. At the



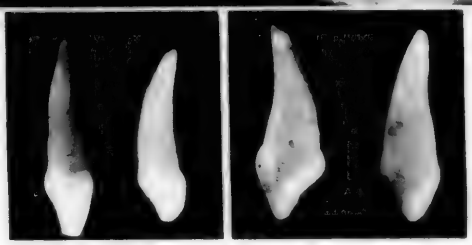


Fig. 31 (Upper) *Fig. 32 (Lower)*

Fig. 31. Right lateral view of human skull of about 5½ years of age with outer or buccal plates of bone removed with exception of narrow strips of bone at crest. The roots of the deciduous teeth and developing crowns of permanent teeth are disclosed. I, II—roots of deciduous maxillary first and second incisors respectively. 1, 2, 3—crowns of permanent maxillary first and second incisor, and canine crowns respectively. 3—crown of mandibular permanent canine tooth.

Fig. 32. Human deciduous anterior teeth showing curvature of apical regions of roots in labial direction.

beginning of hard tissue formation, the phenomenon was exactly the same as was demonstrated for the developing deciduous crown germs—the growing follicle has resorbed the bone surrounding it, and enlarged the space to the ultimate size of the future crown; proliferation of the enamel organ epithelium has progressed eccentrically in all directions as well as deeper within the jaw bone. The crest of bone at that stage was in the region of the present incisal margin of the tooth. The body of the mandible continued to grow vertically by addition

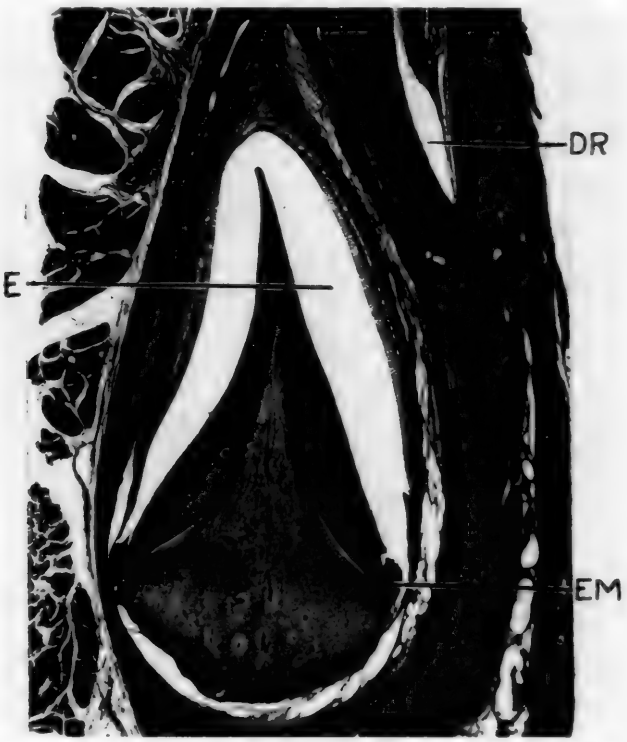


Fig. 33. Histologic sections showing anatomic relation between crown of the permanent mandibular first incisor and the apical end of the root of the deciduous mandibular first incisor. E—calcified enamel; EM—enamel matrix; DR—apical end of the deciduous root of a mandibular first incisor tooth.

of bone at the crest. The crown of the deciduous canine tooth went through similar stages of formation and completion. With the beginning of root formation the tooth started its migratory movement in a vertical direction, the epithelial sheath remaining in a relatively fixed position. The deciduous tooth eventually erupted in the oral cavity, its crown gradually outdistancing its relation to the permanent canine crown, which is still in its original fixed site.

An anatomic detail which has been given little or no consideration is concerned with the morphology of the roots of all the deciduous anterior teeth (1).

The apical regions of their roots (*fig. 32*) are turned labially. This characteristic morphology helps to support the evidence that the crown germ develops in a fixed site.

The positional relationship between the apical end of the root of the deciduous maxillary incisor and the crown of the permanent mandibular incisor tooth is shown in histologic sections, *fig. 33*. By the time the crown of the permanent tooth has completed formation and is well on its way to maturation, the deciduous tooth is well advanced in its eruptive movement. The apical region of its root forms in a direction away from the labial aspect of the crown of the permanent incisor, thereby accommodating its direction of growth to the fixed site of the crown of the permanent incisor tooth.

PART IV. CHANGING LATERAL RELATIONSHIPS DURING GROWTH

All the discussion has been essentially on the vertical relation of teeth and jaw bones during growth. A detailed study of the lateral relations between teeth and bone has to be reserved for a later presentation. There are however, one or two general statements which should be included. One of the principles presented in this study is that no migration of the crown germ occurs in any direction until the enamel of the crown is completely formed and calcification is well advanced. This principle also holds true for migration of the developing crown of the tooth in mesial, distal, labial, or lingual direction.

A horizontal section (*fig. 16*) of the maxillary deciduous anterior teeth in embryonic stage showed the crowding between the developing first and second incisors, and canine crown germs, with the maxillary deciduous second incisor in a lingual relation to the first incisor and canine crown germs. The lingual position of the deciduous second incisor is still in evidence at the birth stage, as demonstrated in *fig. 22B*, which showed the crypts of the anterior teeth in the maxilla. The proper positioning of the second incisor occurs as the growth of the bone widens the perimeter of the anterior curve of the arch. The processes are synchronized so that the second incisor does not migrate to its normal position in the arch until the crown of the tooth has completed its formation.

The synchronization of bone growth with normal tooth positioning is well illustrated by the permanent first and second incisors and canine teeth. The original site of development is different for each one of these teeth. It has been established that hard tissue formation of the permanent maxillary second incisor does not begin until at about 1 year of age, 7 or 8 months after the beginning of hard tissue formation of the permanent canine crown germ.

The explanation for this phenomenon is to be found in the synchronization of the interrelated growth processes. If we refer back to *fig. 31* we can observe that the apices of the roots of both the deciduous maxillary incisor teeth are within the mesiodistal diameter of the crown of the permanent first incisor. There is no room for the development of the maxillary second incisor crown germ. Growth of the jaw bone has to take place in anticipation of the developmental process of the second incisor crown germ. Therefore, the timing of the development of the maxillary permanent second incisor is appreciably delayed to syn-

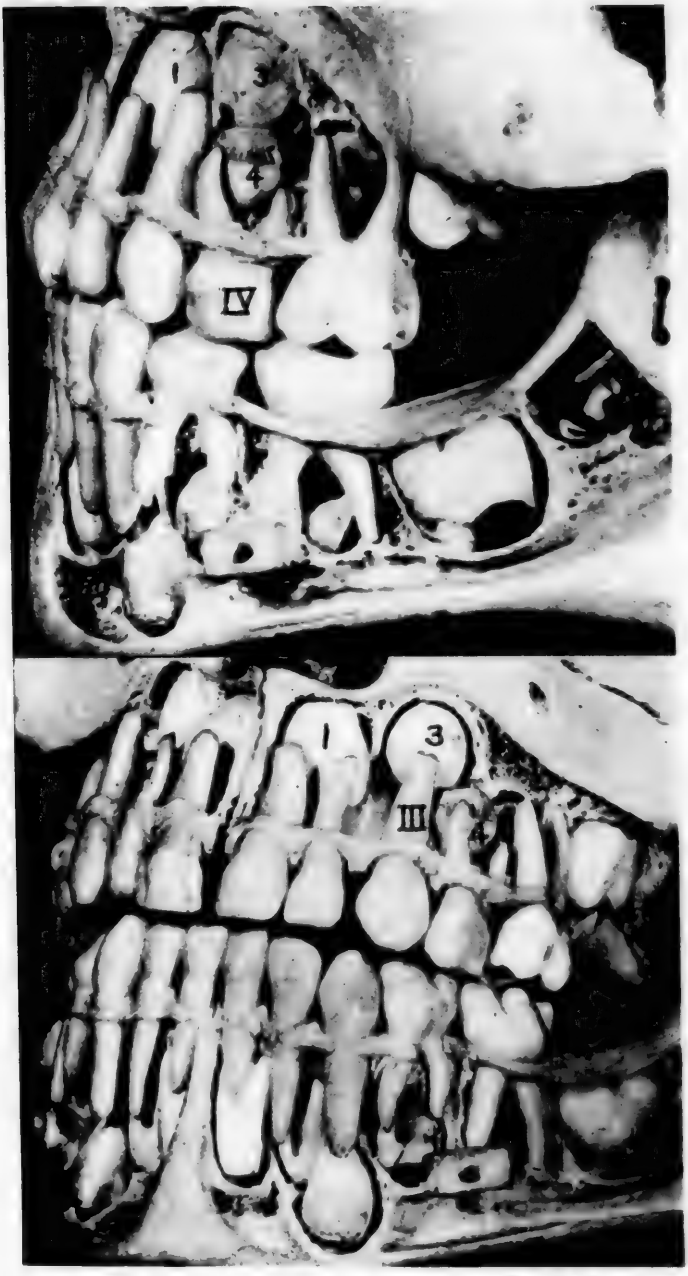


Fig. 34 (*Upper*) *Fig. 35* (*Lower*)

Fig. 34. Left lateral view of human skull of about 5½ years of age with buccal plates of bone removed with exception of narrow strip at crest. Roots of deciduous teeth and the crowns of developing permanent teeth are disclosed. 1—crown of permanent maxillary first incisor; 3—crown of permanent maxillary canine tooth; 4—crown of maxillary first premolar tooth; IV—maxillary first deciduous molar tooth.

Fig. 35. Frontal view of same skull shown in *fig. 34*. Roots of deciduous teeth and crowns of permanent dentition are disclosed in normal anatomic relation between permanent canine crown (3), and deciduous canine tooth (III). 1—crown of permanent maxillary first incisor; 4—crown of maxillary first premolar tooth in mesial direction.

chronize with the bone growth. Even at this 5½ year stage there is still insufficient space between the permanent maxillary first incisor and the canine crowns. They are still in comparative close proximity and the permanent second incisor crown is in lingual direction.

Photographic artifacts (5, 6) are responsible for the erroneous views regarding the lateral migration of developing crowns. *Fig. 34* presents a lateral view of a skull with the outer plates of bone removed. In this photograph the permanent canine, the first premolar, and the deciduous first molar teeth appear directly over each other. This is a characteristic photographic or x-ray artifact; it is readily explained by the law of refraction. *Fig. 35* is another view of the same skull with a central ray directed at right angles to the canine teeth. The permanent maxillary crown now appears in correct relation to the deciduous canine tooth; whereas, the developing crown of the first maxillary premolar tooth appears in a mesial relation to the deciduous first molar tooth.

CONCLUSIONS

The growth and the development of the crown of the tooth must be sharply differentiated from the development of its root. There is no evidence of the epithelial sheath, the outlining organ of the root, until the formation of the enamel matrix is well advanced. The enamel organ is entirely concerned with the formation of enamel; and the loop of continuity between the inner and outer enamel epithelium is the future cervical region of the crown.

The dental follicle is both hero and villain of this story. It is intimately associated with the growth and the development of the crown germ. Like the foetus, the crown germ during its growth and development is a parasite, causing destruction of its host, the surrounding jaw bone, through the invasion of the connective tissue follicle. In this way, the space in the bone is steadily enlarged, and into this space the epithelium of the enamel organ grows in all directions, including into the depth of the jaw bone. This process continues until the follicle forms sufficient space for the ultimate size of the crown of the tooth. The follicle then stops growing and is gradually eliminated by compression through the superimposition of additive layers of enamel matrix. In the final process the peripheral surfaces of the crown lie in proximation to the bone. The remains of the follicle, as is well known, form the periodontal membrane and the cementum of the root. The dental follicle may also be regarded as the placenta of the devloping crown germ. Rich in blood vessels, it undoubtedly serves as the direct source of nutrient supply to the enamel organ.

As long as the follicle is growing the adjacent surrounding bone is being resorbed. When the follicle stops growing the surrounding adjacent bone is in a period of rest until the crown of the tooth is virtually completed. Then the bone becomes active again, particularly in the fundus, where apposition of bone is in evidence; and the crown begins its migratory movement. I believe that this is the essential explanation for the phenomenon of eruption. The mechanism described by Sicher (25) may well function as the trigger action in this connection. At first, the movement is slow because of the resistance of bone at the crest and

the tough fibrous gingival tissue, but as soon as the incisal and occlusal surfaces of the tooth appear in the oral cavity the resistance has been eliminated and the familiar arrangement of bone trabeculae is found at the fundus, indicating more rapid movement. In the normal physiologic process the epithelial sheath is a relatively fixed point, particularly in the period of slow migration before the crown makes its appearance in the oral cavity. In the more rapid phases of vertical migratory movement, the forming root end moves away from the fundus. When, however, vertical migration is inhibited the root grows deeper in the jaw, the fundus being gradually resorbed. Kronfeld (20) presented the histologic evidence, illustrating this phenomenon with a mandibular premolar tooth. Kronfeld, however, believed that the growth of the root deeper in the jaw bone was one of the phases of the normal growth process. He overlooked the fact that the mandibular premolar was crowded between the adjacent teeth and vertical migration was inhibited. This phenomenon is necessarily associated with all impacted teeth.

In a previous presentation (10) I demonstrated the relation of growth of the length of the ramus to the development of the dental height. The evidence showed that a retardation in the length-growth of the ramus failed to increase the intermaxillary space and inhibited the eruption phenomena of the teeth as well as the vertical growth of bone at the crest of the maxilla and the body of the mandible. Thus, the force of eruption is accorded secondary place in increasing the vertical dimension of the dental height; the length-growth of the ramus is the primary factor. Under these circumstances the inhibition of eruption phenomena results in the continued growth of the roots deeper within the jaw bone.

It must be recognized that the jaw bone has its independent pattern of growth which is in no way dependent upon the pattern of growth and development of the teeth. *Fig. 36* presents a series of views of anatomic dissections of the human embryo at 7 months. 'A' and 'B' are frontal and lateral views, respectively, with the maxillary left lip and part of the cheek cut away to disclose the jaws. The location of the crest of the jaw bone is at some distance below the ridge line. Above the crest of bone in the direction of the oral cavity is soft tissue, in the body of which part of the dental follicle and part of the developing crown germ are found. The pattern of bone growth goes on continuously at the crest, increasing its vertical dimension, and simultaneously outward in the direction of the lips and cheek.

This pattern of bone growth proceeds, regardless of whether or not teeth are developing within the jaw. The evidence showed that even at the birth stage the crest of the bone had not grown sufficiently to cover the occlusal or the incisal surfaces of the developing crowns of the teeth; but at the 9 months stage a definite layer of bone is found all along the crest. Bone continues to be formed along the crest, gradually increasing the vertical dimension of the body of the mandible and the dental process of the maxilla. The crown germ develops in its fixed site and as bone is added along the crest the developing crown appears deeper within the jaw bone. At the $5\frac{1}{2}$ years stage the location of the incisal

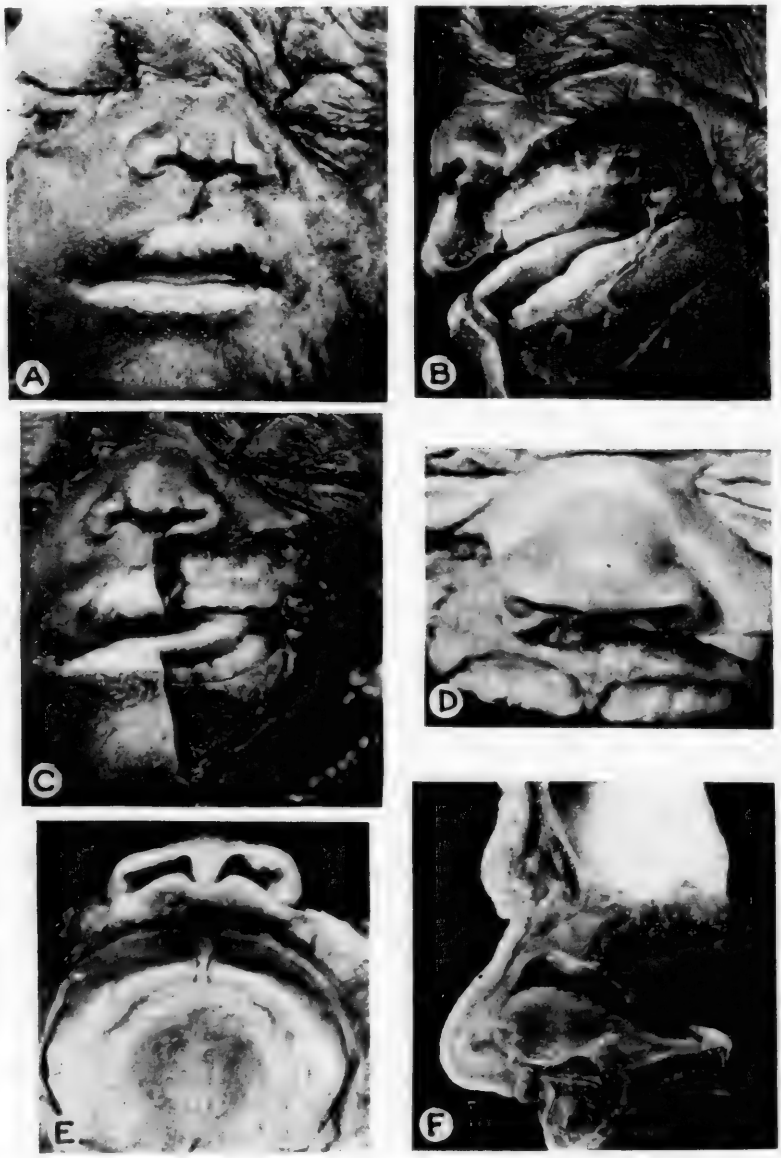


Fig. 36. A—frontal view of human foetus about 7 months. B—lateral view of same foetus with part of the lips and cheek dissected away. C—frontal view of same foetus; the left lips and cheeks dissected away disclosing crest of maxillary bone. Note segmentation of gingival pad resembling contour of underlying crown germs. D—Anterior view of human foetus with lips and cheeks dissected away showing crest of premaxillary bone. Anterior gingival pads in arrangement of segmentation resembling general morphology of anterior teeth. E—Palatal view of maxilla. Note crest of bone and segmented gingival pads. F—Sagittal view of human foetus at sagittal plane. Note relation of maxillary incisor crown germ to floor of nose.

surface of the mandibular permanent canine crown is considerably removed from the crest of bone. This is due to the continuous growth of bone in the vertical dimension, the crown having developed and remained in its original fixed site.

The concept that the alveolar process is a separate entity which grows under the influence of developing teeth (4) is, I believe, unjustified. The alveolar bone is, to my mind, part and parcel of the jaw bone, and the septa of bone designated as alveolar bone are thin only because the teeth occupy the area of the jaw bone between them. In cases of anadontia the growth of bone occurs along the crest, increasing the vertical dimension of the body of the mandible and the dental process of the maxilla. The normal adult dimensions are attained without the presence of alveolar bone.

A retardation of bone growth will necessarily interfere with the proper positioning and arrangement of teeth. This must be differentiated from the general belief that a retardation of bone growth will interfere with the growth and the development of the teeth. This supposed interrelation between bone growth and tooth development is non-existent. Teeth grow and develop normally, regardless of whether there is retardation of bone growth. Only their normal positions and arrangements are disturbed. Even the eruption phenomenon is rarely disturbed except in posterior teeth, due to the retardation of the length-growth of the ramus. The frequent lingual eruption of the permanent incisor or the premolar teeth is indicative of retardation of growth of bone; and these teeth erupt in the position of their original sites of growth and development. I believe, therefore, that the explanation for a large group of cases of malocclusion is to be found in a retardation of growth of the jaw bones. Such retardation need not necessarily be of a permanent nature. The important factor is the proper synchronization between bone growth and tooth development. There are periods during growth and development when the timing factor is more critical than at others. These are the periods of accelerated growth intervals of bone. This will require additional investigation.

SUMMARY

1. The development of the crown of the tooth must be sharply differentiated from the development of its root. 2. The developing tooth, in its stages of development preceding the formation of its root, should be designated as the crown germ. 3. The locus of initiation of the crown germ varies with different teeth of the dentitions. 4. Wherever the locus of initiation of a particular tooth may be, the crown germ continues its development in that particular site without bodily shifting its location until the crown of the tooth is completed. 5. Instead of changing its position, the crown germ grows eccentrically in all directions, according to its pattern. Its vertical dimensions are increased largely by proliferation deeper within the jaw bone, and partly by apposition of enamel increments in the incisal or occlusal regions. 6. The dental follicle is a very important factor in the growth process of the crown germ. Surrounding the outer enamel epithelium, it grows eccentrically in all directions in anticipation of the growth of the crown germ. 7. Growth of the follicle continues until the maximum

space for the completed crown of the tooth is attained. 8. As the follicle grows it steadily resorbs the bone directly adjacent to it. This occurs in all directions including the fundus. 9. The concept that the crown germ moves away from the fundus as it increases its vertical dimension during growth is therefore incorrect. 10. The body of the mandible and the dental process of the maxilla increase their dimensions largely by apposition of bone along the crest. 11. In the embryologic stages and even at the birth stage, there is no evidence of bone along the crest covering the incisal or occlusal surfaces of the crown germs. 12. At the 9 months old period bone is present all along the crest covering the occlusal surfaces of the developing crowns. The addition of bone along the crest, increasing the vertical dimension of the body of the mandible and the dental process of the maxilla, creates the illusion that the developing crowns migrate in the jaw bone. 13. The growth of the crowns of the teeth has its individual pattern as has the growth of bone. They are independent of each other. 14. A retardation of bone growth may influence the positional arrangement of teeth, but not the growth and development of teeth. 15. Synchronization of the growth of bone and tooth development is the important factor for the normal arrangement of teeth in the arch. 16. Migratory movement of teeth occurs only after the dental follicle surrounding the crown has been reduced, resorption of bone adjacent to the follicle has ceased, and the enamel of the crown appears in close proximation to the surrounding bone. The surrounding bone then becomes active, apposition being noted at the fundus as well as in the peripheral regions, simultaneously with the beginning of migratory movement. This may explain the eruption phenomenon. 17. The concept that sutural bone growth increases dimensions by spreading the bone adjacent to a suture, is untenable. 18. Apposition occurs on the outer surfaces of the bones adjacent to a suture. In this way the outer perimeter is steadily enlarged, maintaining the sutural space as growth occurs at the peripheral ends of the bones adjacent to the suture.

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